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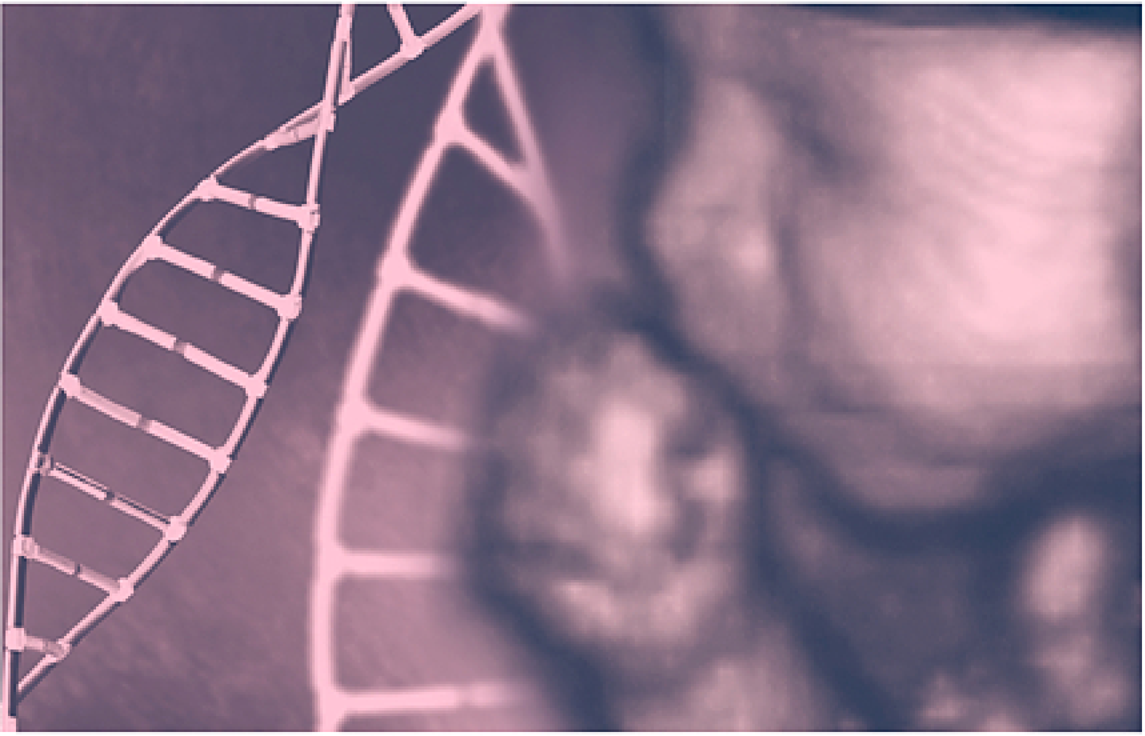

Congenital Diaphragmatic Hernia

Matthew Harting
Kevin Lally
Guest Editors

Seminars in
PERINATOLOGY

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Congenital Diaphragmatic Hernia

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Introduction

Congenital diaphragmatic hernia (CDH) is a congenital, fetal, neonatal, pediatric, and adolescent anomaly which continues to challenge physicians from a wide range of specialties including maternal fetal medicine, obstetrics, neonatology, surgery, cardiology, pulmonology, and pediatrics. In CDH, early embryologic aberrations alter pulmonary vascular, pulmonary parenchymal, and diaphragmatic development. Subsequently, fetal cardiopulmonary maturation is stifled secondary to compression, a prelude to the trifecta of early postnatal pathophysiological consequences: pulmonary hypertension, pulmonary hypoplasia, and cardiac dysfunction. Multidisciplinary intensive care, including ventilation, extracorporeal life support, surgery, and pulmonary hypertensive pharmacology drive early intervention. For survivors, long-term challenges including gastrointestinal, pulmonary, and neurodevelopmental can persist, though a near universal high quality of life awaits.

Significant strides continue to be made at the basic, translational, and clinical levels, advancing our understanding of the basic anatomy, pathophysiology, and optimal clinical care required to optimize survival and quality of life for these patients. In this edition of *Seminars in Perinatology* experts on CDH from multiple continents have been invited to contribute the most up-to-date information (as of summer 2019) on this disease. Moreover, the collaboration of authors from multiple institutions highlights the overarching collaborative approach embraced by investigators in CDH.

Alexandra Benachi and colleagues explore prenatal diagnosis, imaging, and prognosis, highlighting the importance of the prenatal period and the opportunity that exists before the child is born. They discuss a thorough prenatal assessment, the nuances and techniques of prenatal imaging, and emerging methods of prenatal diagnosis/assessment.

KuoJen Tsao and Anthony Johnson keep the focus on the prenatal period with their review of fetal intervention, focusing on fetal tracheal occlusion. By the time children with CDH are born significant pathologic progression renders severe disease extremely difficult to manage. These authors, along with other highly select experts in fetal intervention internationally, are working to reverse this disease before the child is born and before the pathology reaches extreme levels. In their review, they address the rationale for fetal intervention, the importance of accurate prenatal prognostication, the development of preclinical evidence for tracheal occlusion, previously conducted clinical trials in tracheal occlusion, the nuances of procedures in fetoscopic endoluminal tracheal occlusion, and the current, ongoing, international TOTAL trial.

Tim Jancelewicz and Mary Brindle tackle the range of prediction tools, both pre-and postnatal, which have been published and are currently being used in CDH. As our therapeutic armamentarium expands, yet our recognition of the value of standardization evolves, leveraging accurate prediction of severity to guide optimal deployment of intensive therapies stands to be one of the most important contributions to clinical care over the next decade. These authors address both prenatal and postnatal prediction tools, and provide an overarching guideline for the clinician when considering how to best apply such prediction tools to clinical practice.

Moving into the postnatal, therapeutic realm, Yigit Guner and coauthors address the use of extracorporeal life support (ECLS) or extracorporeal membrane oxygenation (ECMO) in the management of CDH. They cover the history of ECMO, predicting the need for ECMO, EXIT to ECMO, indications/contraindications, cannulation challenges, and CDH survival with ECMO support. Although indications and specific uses continue to evolve, ECMO remains a critically important tool in the management of the most severe or high-risk CDH patients.

Congenital diaphragmatic hernia-associated pulmonary hypertension (CDH-PH) is a cornerstone in the pathophysiological evolution of this disease. The unique aspects that define pulmonary hypertension, the molecular origins, the anatomy and histology, the diagnostic features, the natural history, current management, and emerging therapies are addressed in this review from Matthew Harting and Vikas Gupta. A breakthrough in our ability to manage this process may drastically change the outcome for children and families with CDH.

Neil Patel and colleagues provide an exciting overview of the most novel concept emerging in our understanding of congenital diaphragmatic hernia: cardiac dysfunction. Evidence would suggest that cardiac, and in particular ventricular, dysfunction is a key contributor to CDH pathophysiology. These authors review the pathophysiology of ventricular dysfunction in CDH, address the role of both the left and right ventricle, summarize the association between cardiac dysfunction and outcome, outline key principles for assessment

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and management of cardiac dysfunction, and provide a glimpse at future investigation into this topic.

Wendy Chung and coauthors provide a thorough overview of the influence of genetics in CDH. Despite the fact that only approximately 30% of CDH has a known, underlying genetic association today, this team is at the forefront of further investigation into the role and association of genetics and CDH. They address chromosomal anomalies known to be associated with CDH, monogenic syndromes associated with CDH, previously identified genes associated with CDH, and the importance of genetic counseling for families.

Richard Keijzer and colleagues provide an elegant overview of the current basic and translational understanding and approaches to investigating CDH. This type of work forms the foundation of our understanding of CDH and is critically important if we are to continue to identify novel approaches for attacking this disease process. They address advances at the level of the gene, at the epigenetic level, at the protein level, and the translational approaches that are the result of investigational efforts.

Finally, Laura Hollinger and Terry Buchmiller delve into the issue of survivorship in CDH. They offer an overview of the challenges that modern CDH survivors face and include a risk-stratified algorithm as a guideline for multi-disciplinary long-term follow-up. Specifically, they address the role and components of a long-term follow-up clinic, specific areas of follow-up including cardiopulmonary, neurodevelopmental, gastrointestinal, musculoskeletal, and nutritional, as well as needs that are specific to surgeons and the impact on the entire family. They also address unique morbidities associated with prenatal intervention in the transition of care, as these adolescents become young adults.

CDH is a multi-system, multi-specialty disease with multiple phases of care including prenatal, acute postnatal, and long-term. Advances at many levels are changing our understanding of the basic mechanisms of disease and augmenting our interventional approaches. International collaborative efforts continue to drive the most significant advances in this relatively rare disease. With a solid historical foundation and exciting breakthroughs occurring on a myriad of levels, the future of CDH research is poised to improve the lifetimes of children and families touched by this disease.

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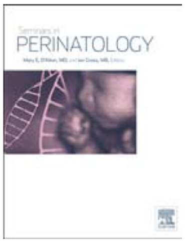

Prenatal diagnosis, imaging, and prognosis in Congenital Diaphragmatic Hernia

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ABSTRACT

Antenatal ultrasound screening identifies more than 60% of Congenital Diaphragmatic Hernia (CDH) cases and provides the opportunity for in utero referral to a tertiary care center for expert assessment and perinatal management. Prenatal assessment of fetuses with CDH has tremendously improved over the past ten years. The outcome may be predicted prenatally by medical imaging and advanced genetic testing. The combination of lung size and liver position determination by ultrasound measurements and MRI are widely accepted methods to stratify fetuses into groups that correlate not only with neonatal mortality but also with morbidity. Notwithstanding this, prediction of persistent pulmonary hypertension of the newborn still needs to be improved.

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Introduction

The prenatal diagnosis of congenital diaphragmatic hernia (CDH) has improved worldwide since the introduction of prenatal screening ultrasound (US) and today, two thirds of cases are detected by the second trimester.[1] Ideally, this should lead to referral to a tertiary center given that planning birth of affected fetuses in a center with experience in the perinatal management of CDH, with standardized neonatal management in an optimal neonatal care intensive unit (NICU) and pediatric surgery services, improves outcome.[2,3] Specialized assessment is also needed to rule out associated anomalies, as in up to 40% of cases CDH are not isolated, and the outcome is codetermined by the severity of associated conditions.[4] Personalized prediction of disease severity and prognosis[4] by genetic testing and advanced imaging (US and MRI) is mandatory to counsel parents, but also to discuss the potential of antenatal management, such as termination of pregnancy or fetal therapy, which today is available within the framework of clinical trials.[5]

Prenatal assessment

Genetic testing

For exhaustive diagnostic evaluation and accurate counseling, genetic testing should be completed. There is no specific

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cytogenetic anomaly which has been associated with CDH, but a causative cytogenetic aberration is detected in 2% to 33% of patients.[6,7] Even if many of the most common chromosomal aberrations can be diagnosed by conventional karyotyping, an additional 6 to 9% have clinically relevant copy number variants identifiable by array comparative genomic hybridization.[6,8] In a recent study, targeted resequencing has enabled the identification of previously unidentified genetic causes in 10% of archived samples.[8]

Increasingly, more cases are diagnosed in the first trimester and therefore choriovillous sampling (CVS) is then offered. Although array comparative genomic hybridization can be performed on CVS, one has to remember that the mosaic distribution of the supernumerary isochromosome 12p (Pallister–Killian syndrome) greatly increases the risk of misdiagnosis.[9,10] Therefore, an amniocentesis with array comparative genomic hybridization on non-cultured cells is recommended.[11] In case of multiple associated structural anomalies, the search for specific mutations and syndromes is obviously recommended.[12]

A more detailed overview on genetic anomalies associated to CDH is included in this edition of the *Seminars* or we refer to the specialized literature.[12,13]

Prenatal imaging

Accurate prediction of outcome is crucial given the fact that fetuses with the worst prognosis may benefit from prenatal intervention, currently including tracheal occlusion, in the clinical trial setting. Personalized prediction is made based on the isolated nature of the defect, its side, estimation of lung size, and presence and amount of liver herniation, which can also be predicted with stomach position or measured by MRI[14–17] (Table 1–3).

Lung size

Measurement of lung size is the most logical and direct approach to assess hypoplasia and subsequently predict neonatal outcome. The lung–to–head ratio (LHR), first described by Metkus et al.,[15] provides an indirect estimate of the size of the lung contralateral to the hernia, normalized for the head circumference. It is typically measured by 2-dimensional ultrasound at the level of the 4-chamber view (Fig. 1). The most accurate methods for lung measurement are by tracing the lung contours or by the long axis method.[18]

Table 1 – Imaging options and measurements allowing an exhaustive assessment of CDH.

US	MRI
LHR o/e[18]	TFLV o/e[14]
Liver position	LiTR[29]
Stomach grading[17]	Stomach grading[31]
	3D defect's size[70]

LHR: Lung to Head Ratio; TFLV: Total fetal Lung Volume; LiTR: Liver into Thorax Ratio.

Table 2 – Prognosis of Left CDH with liver UP correlates with LHR observed/expected measured at US evaluation.

Left CDH, liver UP	LHR o/e	Survival
Very severe	<15%	<5%
Severe	16–25%	20–30%
Moderate	26–45%	40–60%
Mild	>45%	95%

Table 3 – Prognosis of Left CDH depending on liver position and TFLV (Total Fetal Lung Volume) by MRI.

	TFLV o/e	Survival	
		Liver UP	Liver DOWN
Very severe	<15%	12%	40%
Severe	16–25%	40%	85%
Moderate	26–45%	65%	85%
Mild	>45%	75%	85%

LHR measurement was initially only used in the late second trimester and values under 1.0 were considered predictive of poor outcome. However, the LHR changes over gestation as the lung area grows more rapidly than the head circumference. Correction for gestational age by expressing the LHR of the index case as a function of what is expected in a gestational age matched control (observed/expected LHR or o/e LHR) is therefore used. The formulas for calculating the o/e LHR are embedded in a freely available web-based calculator[5] (www.totaltrial.eu). In left sided CDH (LCDH), when o/e LHR is <25%, hypoplasia is considered severe. The next severity group (25%<o/e LHR<35% irrespective of liver position, or 35%<o/e LHR<45% with liver "up") is (a bit misleadingly) categorized as "moderate" as survival in that group is limited to 50–60% and survivors have significant morbidity.[18]

The o/e LHR has been shown to be an independent predictor of postnatal survival[18] both in LCDH and RCDH, and of some

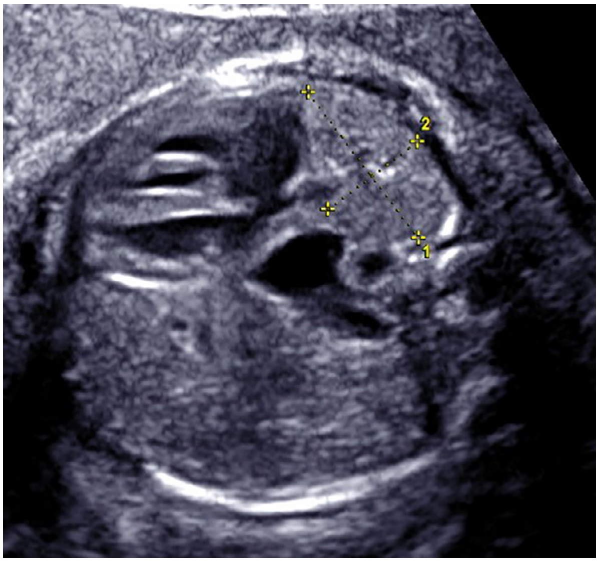

Fig. 1 – Measurement of the contralateral lung in a fetus with left congenital diaphragmatic hernia at the level of the four chambers view of the heart, using the longest axis method.

short–term morbidity indicators.[19,20] The o/e LHR is therefore now widely accepted as a prenatal tool to counsel parents and is used to select patients for fetal therapy, such as the TOTAL (Tracheal Occlusion To Accelerate Lung growth) trials.

Other 2D ultrasound methods to assess lung size, such as the lung to thorax ratio[21] and the quantitative lung index[22] have also been proposed, but they are less validated hence not discussed here. Lung volume estimated by 3-dimensional US also correlates with neonatal outcome, yet is not better than O/E-LHR, and volume estimation may be difficult on the ipsilateral side.[23,24]

Liver position evaluation: direct and indirect methods

In addition to measuring the o/e LHR, the severity of CDH is estimated based on the binary variable, hence, either “up” (in the thorax) or “down” (confined to the abdomen) liver position.[25,26] In left sided cases, this factor is an independent predictor next to lung size (as o/e LHR) according to some studies.[14,25] However, in right–sided CDH, the liver is nearly always herniating through the defect, hence it has no predictive value.[27]

Werneck-Britto et al.[28] described the liver-to-thoracic area ratio (US-LiTR) by 2D US, measured on the standard 4-chamber view and demonstrated a predictive accuracy for neonatal outcome. As the echogenicity of the liver is very similar to that of the lung, it can be difficult to assess the liver position or its extent into the thorax and therefore it is more easily evaluated prenatally by MRI. Cannie et al. proposed to measure the ratio of the volume of liver to that of the total chest (liver-to-thoracic volume ratio, LiTR), whereas Lazar et al. calculated the percentage of liver that is into the fetal chest (MRI-% Liver herniation).[29,30] The amount of liver into the chest seems to be a predictive marker of postnatal survival in CDH.[29–32]

However, it is far easier to determine the stomach position rather than quantify liver herniation, as the stomach is anechoic, whereas liver echogenicity is close to or identical to that of the lung.[33] Stomach position was suggested as an indirect method to estimate severity of the disease and a prognostic indicator previously in 1992.[34] Kitano et al.,[16] proposed a simple stomach grading correlated to the degree of herniation (in 4 steps) on coronal sections of the thorax using MRI. He demonstrated there is a relationship between stomach position and intrathoracic liver position. More recently, we introduced a 4-step grading system using the standard 4-chamber views on US, hence facilitating its reproducibility, and correlated this with the proportion of intrathoracic liver LiTR determined by MRI in left–sided CDH (Fig. 2).[35] In that series, the position of the stomach correlated well with postnatal outcome, independent from the o/e LHR.[17]

Most prenatal management centers use a combination of the position of the liver and the o/e LHR to stratify patients into different groups with increasing degree of pulmonary hypoplasia and corresponding risk of mortality. Lung size and liver herniation can also predict short term neonatal morbidity in terms of assisted ventilation, need for supplemental oxygen, need for patch repair and time to full enteral feeding.[36–38] These factors, in some studies, also correlated with persistent pulmonary hypertension of the newborn.[37,39]

Hernia sac

A particular anatomical form of CDH is when a hernia sac, formed by a peritoneal layer covering the digestive organs herniated in the thorax, is present. A number of studies have focused on the association between the presence of a hernia sac in CDH and morbidity and mortality.[40–45] The percentage of CDH cases with a hernia sac ranges between 14.2%[42] and 25.7%.[40] Several authors report a better survival rate for CDH-neonates with a hernia sac, but difference in morbidity is still controversial.[40–45]

Zamora et al. described 3 prenatal signs revealed by MRI: (1) meniscus of lung posterior or apical to the hernia contents, (2) encapsulated appearance of hernia contents, and (3) presence of pleural fluid outlining a sac from above.[41] They found that these MRI signs were correlated very specifically with the presence of a hernia sac. However, Bouchghoul et al., evaluated those signs retrospectively by ultrasound; the positive predictive value of the 3 signs was low, but the negative predictive value was high.[44]

The efficiency of these prenatal signs is currently not sufficient to allow their use in prenatal counselling. Further prospective studies are necessary to evaluate those prenatal ultrasound signs of hernia sac in CDH.

Imaging for associated structural anomalies

The prevalence of associated structural anomalies ranges widely in different studies, being higher where stillbirths or cases undergoing termination of pregnancy are included.[46,47]

A wide spectrum of structural anomalies associated with CDH has been reported in the literature. Cardiovascular defects are the most common anomalies associated with CDH and are found in about one third of all CDH patients and in 15% of non-syndromic cases. Therefore, all patients with CDH should undergo high–quality fetal echocardiography.

Finally, intrafetal fluid effusions are a relatively common ultrasound finding in CDH fetuses, observed in up to 5% and 29% of left– and right–sided CDH cases, respectively. One series did not observe a correlation with poorer outcome.[48]

CDH-associated pulmonary hypertension (CDH-PH)

Neonatal death in CDH infants is a consequence of pulmonary hypoplasia, but also of CDH-PH.[49] CDH-PH is believed to have a morphological and functional basis. Smaller and fewer pulmonary arteries lead to increased pulmonary vascular resistance along with increased vasocontraction.

Although fetal volume estimation is now feasible by 2D-ultrasound, 3D-ultrasound or MRI determinations, their use for the prediction of the occurrence and severity of CDH-PH is still part of research programs. This represents a weak point of prenatal prognostic assessment.

The logical approach to assess lung vascularization is by direct measurement, which has been attempted by a variety of techniques.[23,50–56] These measurements are not easily reproducible, and their added predictive value remains to be proven as shown in a recent meta-analysis.[57] However, prediction of CDH-PH would be desirable, as it correlates with mortality and long-term morbidity.[58]

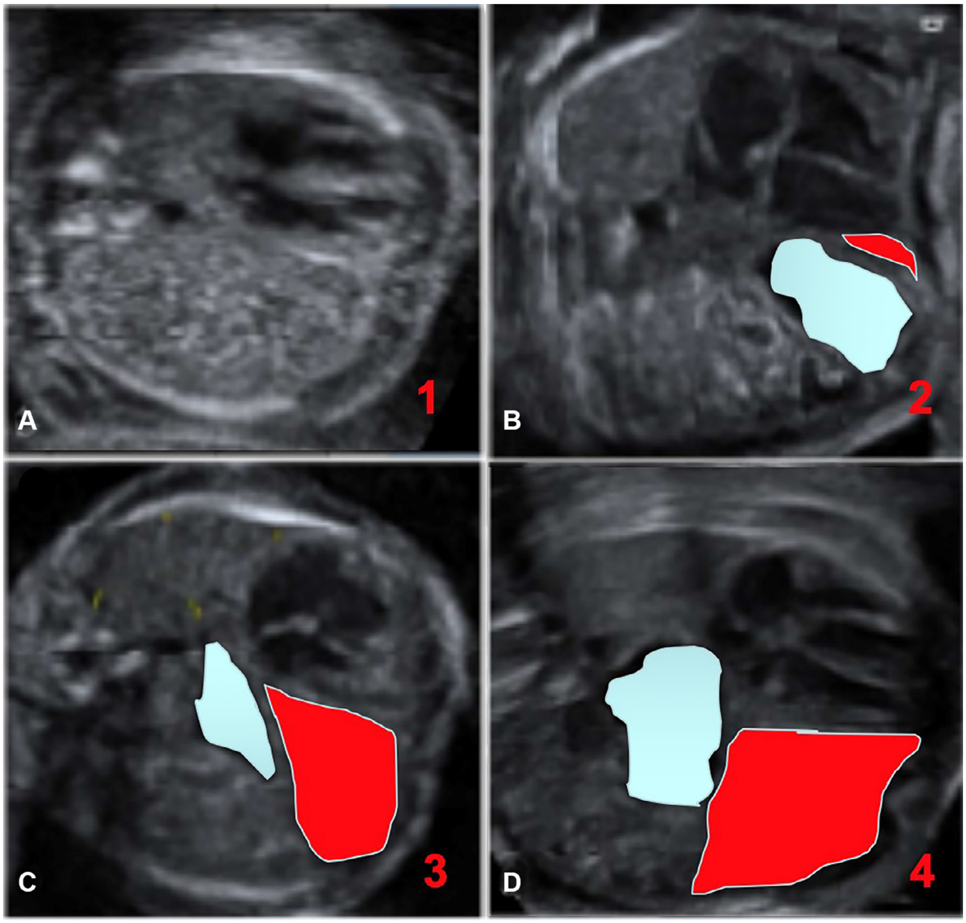

Fig. 2 – Grading of liver herniation based on stomach position. Liver in red and stomach in blue. Grade 1 or abdominal: the stomach is in its normal position hence not visualized in the chest (A); Grade 2 or anterior left in the chest: the stomach is visualized anteriorly at the apex of the heart, in contact with the anterior chest wall (B); Grade 3 or mid–to–posterior left chest: the stomach is not in contact with the anterior left chest wall; yet next to the atrial–ventricular heart valves but with most of it anterior (C); Grade 4 or retrocardiac: most of the stomach is located posterior to atrial–ventricular valves, next to the left atrium of the heart within the right chest (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Magnetic resonance imaging (MRI)

Fetal MRI is widely used in the assessment of fetal lung disorders including CDH and is currently the reference technique for fetal lung volume estimation.[59,60] MRI, with shorter acquisition times, is not limited by maternal habitus, fetal position, or amniotic fluid volume[14] and allows for accurate and reliable measurement of both lungs.[61] Analogous to the o/e-LHR, absolute bilateral volumes are converted to a percentage of what is expected in a normal fetus (observed-to-expected total fetal lung volume; o/e-TFLV)[59] (Fig. 3).

Volumetry can also be used to quantify liver and stomach herniation. The degree of volumetric intrathoracic liver herniation has been shown to better predict postnatal survival than the dichotomous assessment or either presence or absence of the liver into the chest.[29,30,62,63] However, due to its wider availability, ultrasound remains in many centres the modality on which decisions regarding prenatal management are based.

Right-sided congenital diaphragmatic hernia

Right-sided diaphragmatic hernia accounts for 13–17% of all CDH cases. Right CDH (RCDH) is considered to be overall more severe than left CDH, but this is still debated.[64–67]

Jani et al.[18] and De Koninck et al.[27] documented an overall survival of 44% to 53% respectively. In those studies, the o/e LHR correlated with survival, yet the cut off for severe hypoplasia apparently is higher; when O/E LHR is <45%, survival rate was extremely low. Additionally, liver herniation is not discriminative in RCDH, as it is nearly always present.[27,64] Schaible et al.,[65] reported that right CDH carries a higher risk of long-term morbidity, especially chronic lung disease. Given the rarity of RCDH it will be difficult to get sufficient data to develop a side specific prediction algorithm for RCDH. Right and Left CDH data must not be pooled in clinical studies as the prognostic evaluation and probably the physiopathology could be different between the two diseases.

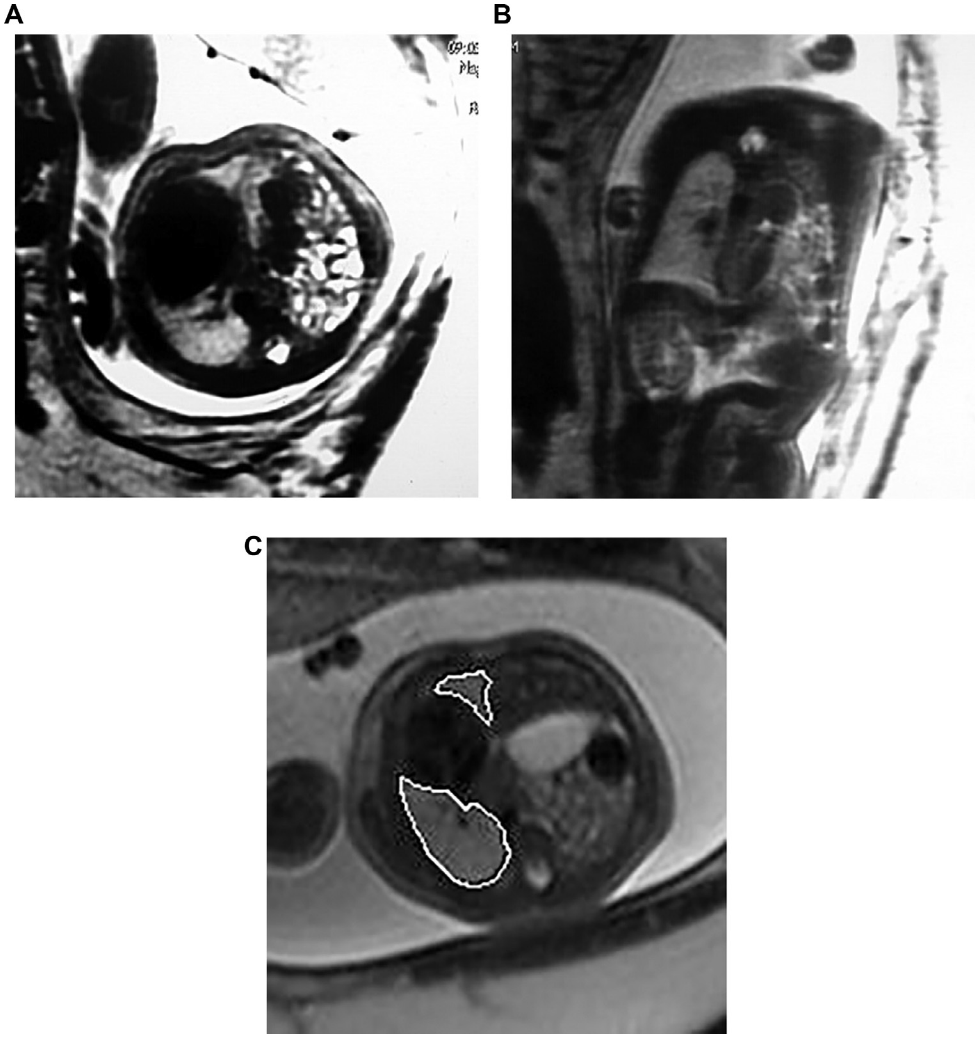

Fig. 3 – MRI assessment of left CDH allows calculation of Total Fetal Lung Volume (TFLV observed/expected). A: axial, B: coronal; C: axial with lung area highlighted.

Future or emerging methods of diagnosis

Further emerging methods of diagnosis are needed in order to better evaluate CDH-PH. As mentioned before evaluation of distal lung vascularization could be the ideal technique. Diffusion MRI has been used as well as quantitative analysis of fetal pulmonary vasculature.[23] Unfortunately, the external validation by other teams has never been published, and therefore is not been considered as been reproducible. Those techniques are also time-consuming preventing them to be used in regular clinical setting.

As defect size has been considered as the only real reliable post-natal marker of morbidity,[68,69] a recent paper has focused on the possibility of evaluating the exact size of the defect by prenatal MRI.[70] Prayer et al. has measured surface areas of the intact diaphragm and of the defect to calculate defect-diaphragmatic ratio.[70] It seems that this ratio correlates with the need for patch at surgery and that MRI-based 3D reconstruction allows location, classification and quantification of the defect.

Conclusion

Today, prediction of neonatal outcome and morbidity is possible based on prenatal imaging. Prenatal sonographic assessment remains heterogenous and should improve, especially in the era of prenatal treatment because not all CDH fetuses will be eligible to receive prenatal investigational intervention (FETO, sildenafil, etc.).[71] Efforts must continue to improve the quality and consistency of prenatal diagnosis and evaluation, in both right and left CDH. Moreover, survivors require multidisciplinary follow-up, and research linking antenatal markers for CDH-PH with long-term morbidity is still needed.

Sources of support

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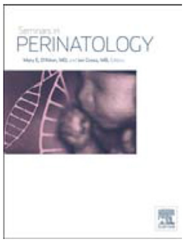

Fetal tracheal occlusion for congenital diaphragmatic hernia

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ABSTRACT

Congenital diaphragmatic hernia (CDH) remains one of the most elusive birth defects to treat. Despite greater knowledge of disease and advances in technology, approximately one-third of CDH children born today still die. Consequently, clinicians and researchers have struggled to find the optimal treatment strategies for CDH. Without further innovations in postnatal treatment, many have focused an antenatal approach to improve pulmonary function. Fetoscopic Endoluminal Tracheal Occlusion (FETO) for CDH has evolved to the bedside after decades of research. While still under clinical investigation, FETO remains a promising adjunct to the treatment of CDH.

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Introduction

Over the past few decades, significant investigative efforts to understand the etiology, natural history, and treatment of congenital diaphragmatic hernia (CDH) have occurred. Although the incidence is fairly rare at 1 to 4 per 10,000 live births,[1] the true frequency of disease is probably closer to 1 in 2000 births when considering the unreported "hidden mortality" associated with stillbirths and deliveries at non-tertiary centers.[2] CDH is a prenatal condition, although rare, can be diagnosed early in pregnancy and well characterized with prenatal imaging in order to increase our ability to prognosticate postnatal mortality.[3,4]

Today, we know that the severity of disease is related to the CDH-associated pulmonary hypoplasia and hypertension (CDH-PH). Although surgical intervention is necessary to repair a CDH, most recent advances have been in non-surgical therapies. Lung-protective ventilator strategies, extracorporeal membrane oxygenation (ECMO), and pre-operative stabilization prior to surgery have all led to a significant decrease in mortality. Current management strategies have produced an overall improvement in survival from 40% to 60 to 70%,[5] including greater than 90% survival for less severe defects.[6] Yet, overall survival among the most severe cases of CDH remains stagnant over time, approximately 50% in most centers in the CDH Study Group.[6] The overall mortality may be even higher considering these data represent a select group of centers with special interest in CDH care.

The stalemate of CDH care has resulted in one of the most costly and lethal fetal malformations,[7] with a cadre of investigators seeking alternatives to current, conventional therapy. With a well-defined natural history and strong correlation between prenatal predictors and outcomes, CDH continues to drive scientific curiosity for antenatal intervention to improve lung function prior to delivery.[8] Today, antenatal interventions

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for CDH exist and have evolved to a minimally invasive approach in the form of Fetoscopic Endoluminal Tracheal Occlusion (FETO). To understand fetal intervention for CDH, this article will address the pathophysiology of CDH, which gives rationale to prenatal intervention, prenatal diagnosis and prognostication of CDH including their challenges, and the current state of clinical trials in FETO.

Rationale for fetal intervention

Fetal lung development occurs in five overlapping stages. However, fetal intervention for CDH targets the physiological derangements during mid-gestation. Pulmonary vessels, respiratory bronchioles, alveolar ducts take shape during the *canalicular stage* which occurs from the 16th to the 25th weeks. Type 1 pneumocytes begin to appear, as well as the precursors for type 2 pneumocytes. Functional respiratory units, known as the acinus consisting of alveoli, alveolar ducts, and respiratory bronchioles, form and multiply along with the pulmonary vessels.[9,10] The *saccular stage* continues from 24th week to term with maturation of the alveolar sacs with maturation of airway dimensions and surfactant synthesis capabilities.[11,12]

Any impedance to normal pulmonary development will simultaneously hinder proper pulmonary vascular development. CDH patients can demonstrate significant ventilation to perfusion (V/Q) mismatching, predominantly due to abnormal lung perfusion.[13,14] Because pulmonary vasculature and alveoli grow concurrently, normalization of pulmonary function can occur, but is most likely due to increased lung volume rather than perfusion.[10,15] This lends some support to the rationale for FETO in promoting lung growth in patients with CDH to improve pulmonary outcomes as well as remodeling of the pulmonary vasculature associated with CDH-PH.

Preclinical evidence for fetal tracheal occlusion in CDH

The current strategy of fetal tracheal occlusion for the treatment of CDH was developed through decades of methodical research in animal models. Led by Harrison and colleagues at the University of California, San Francisco, a series of experiments were conducted that followed guiding principles of fetal therapy: understand the natural history of human disease, re-create the disease state in animals, demonstrate reversibility of the derangements with fetal intervention, and maintain an acceptable risk profile for the fetus and mother.

The first models of fetal CDH were developed in sheep by placing an inflated balloon in the chest to mimic compression by intrathoracic abdominal viscera.[16] Displacement in the thoracic cavity produced hemodynamic instability and impairment of pulmonary growth. When the balloon was deflated, pulmonary development was recovered and the fetal sheep survived to term delivery, suggesting that fetal intervention for CDH may be reversible. Although this model did not mimic the diaphragmatic defect and was conducted late in gestation, it is served as proof of concept that fetal pathophysiology could be created and reversed during gestation.

Next, surgical creation of diaphragmatic hernia was created at 100 days gestation in lambs.[17] Similar impairment of normal lung development was seen. Early attempts at fetal repair of the diaphragm at 120 days gestation resulted in fetal demise secondary to obstruction of the umbilical vein due to reduction of the abdominal viscera. In response, a 2-stage approach was taken, which included an abdominoplasty incision utilized to relieve intra-abdominal pressure. As a result, survival improved to term with responsive lung growth.[17] This strategy was utilized in the initial attempts to repair CDH prenatally in humans. The first cases of fetal intervention for CDH were reported in the early 1990s.[18,19] Although the technical aspects of maternal-fetal surgery were successfully completed, preterm labor and ongoing physiological impairment led to postnatal demise. Consequently, this approach was abandoned for alternatives that would promote lung growth without the significant risk and impact of open maternal-fetal surgery.

The concept of tracheal occlusion to improve fetal lung development by obstructing the trachea and preventing egress of pulmonary fluid to promote pulmonary hyperplasia existed for almost 30 years prior to the first fetal intervention of CDH repair in humans.[20,21] However, it was Wilson and colleagues who first suggested that tracheal occlusion could be used for CDH.[22] In fetal lamb studies with surgically created diaphragmatic hernias, tracheal obstruction expanded the fetal lung, reduced the abdominal viscera, and produced larger and better functioning lungs at birth compared to untreated controls.[8,23–25] As a result, the strategy to promote fetal lung growth shifted to the ‘PLUG’ technique (Plug the Lung Until it Grows).[23,24]

Several models of fetal tracheal occlusion were developed in the mid-1990s.[24,26,27] Foams and plugs failed to provide total tracheal occlusion. Tracheal clips required secondary procedures for removal and tracheal reconstruction. Eventually, vascular occlusion balloons were chosen due to several advantages: avoided secondary surgical procedures, provided total tracheal occlusion while allowing tracheal growth, required a simpler technical approach, and minimized dysmorphism of the trachea. Eventually, with the advent of percutaneous fetoscopic access to the fetus, this strategy could be accomplished without hysterotomy and thus minimizing maternal risk.[28]

Although fetal tracheal occlusion demonstrated increased lung volume, ongoing pulmonary distention had shown significant decrease in the production of type 2 pneumocytes and surfactant production[29–31] with an increase in the risk of PH in CDH-affected neonates after birth.[32] Relieving the tracheal occlusion before delivery exhibited improved pulmonary maturation and increased production of type 2 pneumocytes compared tracheal occlusion maintained until delivery. Nelson et al. demonstrated in the sheep that cyclical occlusion provided optimal growth of the lungs by maintaining growth, while preventing type 2 pneumocyte impairment due to over distension.[33] Consequently, the “plug–unplug“ strategy was implored for fetal balloon tracheal occlusion for the treatment of CDH.

Prenatally-derived prognosis of CDH

One of the key tenets of offering fetal intervention is the ability to accurately prognosticate the severity of the disease.

As such, there has been a significant effort directed over the past two decades in the evaluation of various US and MRI markers to predict neonatal outcomes, primarily pulmonary hypoplasia and CDH-PH. These risk assessments have been utilized to identify the fetus with CDH who may potentially benefit of an *in utero* intervention.

The antenatal imaging strategies have been based primarily on estimation of fetal lung volumes and intrathoracic liver herniation. Metkus et al. were the first to report the use of the lung area to head circumference ratio (LHR) obtained by two-dimensional US as a tool to estimate fetal lung size.[34] In the original report the area of the contralateral lung was calculated using the product of the two longest perpendicular linear measurements obtained in a transverse view of the fetal chest at the level of the four chambered. Others have reported LHR results using two different methods of measuring the contralateral lung size, the anterior-posterior diameter (AP) at the mid-clavicular line by the perpendicular diameter at the midpoint of the AP line and manual tracing of the limits of the lung.[35] The trace method has been shown to be the most reproducible and accurate means of obtain the lung area with two-dimensional (2D) ultrasound (Fig. 1a–c).[35,36]

Alternative methods of calculating lung size have been reported including three-dimensional measurements of the lung, lung-to-thorax ratio and the quantitative lung index. These strategies have not been well validated or have been shown to be less accurate the observed to expected LHR (o/e LHR).[37,38]

The LHR is most predictive when used between 22 and 28 weeks' gestation however, LHR will normally increase with advancing gestation due to the fact there is 2.7 fold in increase in lung growth compare to a 0.7 increase in head circumference from 20 to 32 weeks' gestation.[39] Therefore, LHR is a gestational age–dependent measurement. To adjust for this increase normalizing the LHR for gestational age by using an observed-to-expected LHR (o/e LHR) has become the preferred method for assessing lung volumes in fetal CDH. The predictive value of the o/e LHR measurement has been shown

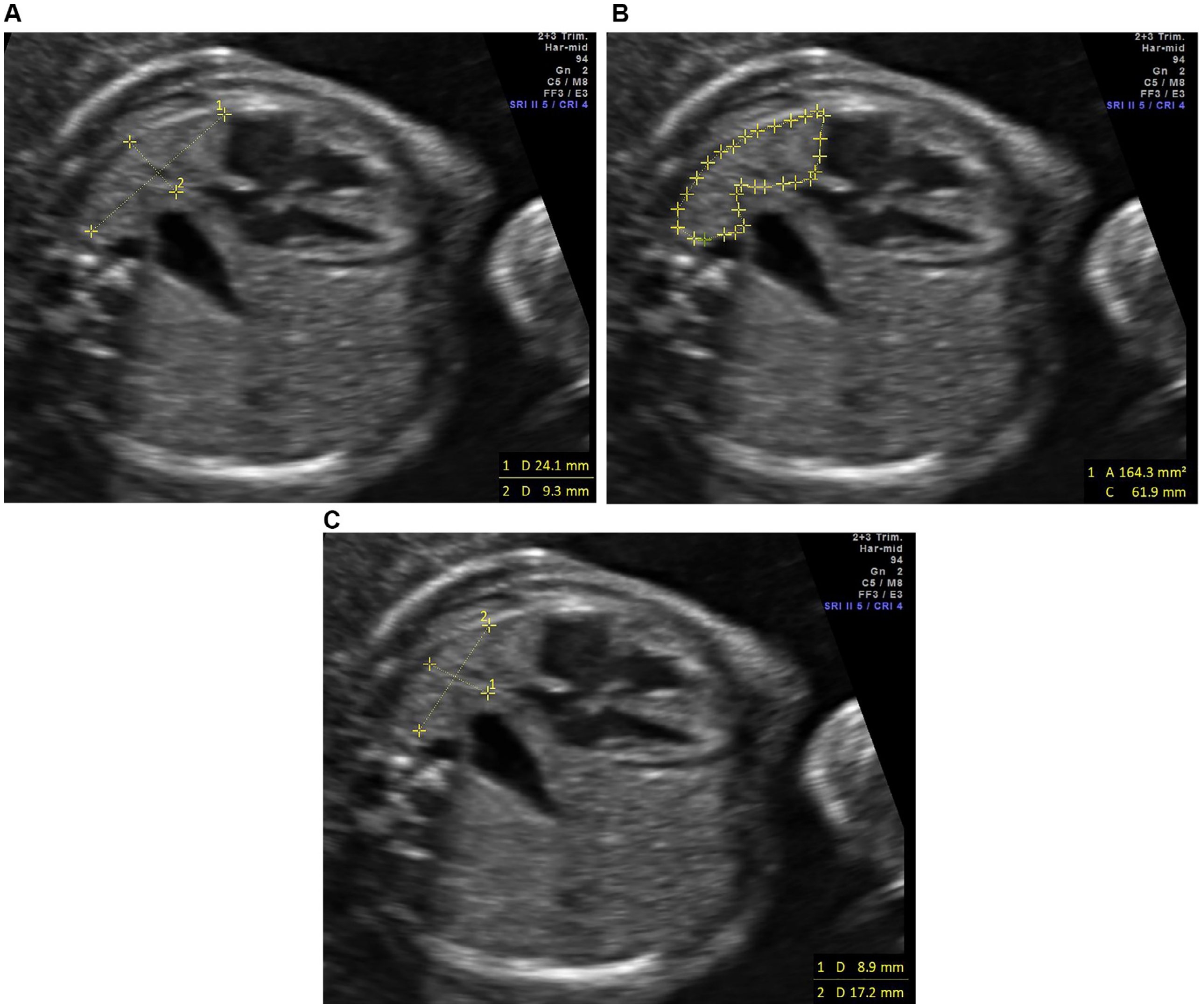

Fig. 1 – Two dimensional ultrasound images showing measurements of the contralateral (right) lung area in the transverse plane of the fetal thorax at the level of the four-chambered heart. (A) longest diameters, (B) trace of lung limits and (C) anterior posterior diameter from the mid-clavicular line.

to be accurate throughout gestation but with a trend toward better prediction later in gestation.

MRI has some distinct advantages over US that make it useful tool in the evaluation of fetal lung volumes and liver herniation in CDH and is becoming the primary technique for evaluating fetal lung volumes in tertiary care centers. There is visualization of the ipsilateral lung that is generally not obtainable by US and less hampered by maternal body habitus. MRI parameters that have been evaluated include total fetal lung volume (TFLV), observed to expected TFLV (o/e TFLV) and percentage predicted lung volume (PPLV), percent liver herniation (%LH) and liver intrathoracic to thoracic ratio (LiTR). As with US, the o/e TFLV, corrected for fetal body weight, has been the most frequently studied to determine fetal lung volumes (Figs. 2a-b).[40]

Liver herniation into the fetal chest is associated with neonatal mortality and long-term morbidity. Intrathoracic herniation is detectable by US or MRI. Many studies have simply reported the herniation as “intrathoracic” or “intrabdominal” without quantitation of the percent herniation. In the meta-analysis by Knox et al., US detected liver herniation into the fetal chest was also found to be a strong predictor of poor perinatal outcome (survival OR:0.32; 95% CI: 0.21–0.49).[41] A later systematic review and meta-analysis of US and MRI by Mullassery et al. found in 710 fetuses that intrathoracic liver herniation was associated with an increased risk of neonatal mortality, 45.4% ($n = 407$) vs 73.9% ($n = 303$), $P < 0.0005$. When 88 cases of fetal intervention removed from the analysis the difference remained significant.[42] Liver herniation has been associated with an increased risk of need for ECMO and chronic lung disease as well.

The recent meta-analysis from Oluyomi-Obi compared the following US and MRI antenatal parameters for the prediction neonatal mortality in CDH; LHR, o/e LHR, TFLV, o/e TFLV, PPLV, and%LH.[43] The study found that the best predictors were o/e LHR, TFLV and%LH. o/e TFLV was slightly superior to o/e LHR with AUC of 0.8 and 0.78, respectively, however this difference resolved when o/e LHR was obtained by the trace method. Survival rates at various thresholds were: o/e LHR < 25%, ranged from 12.5% to 30%) and > 35% were 65–85%; o/e TFLV < 25%, 25-35% and > 35% were 0–25%, 25–69% and 75–89% respectively. Liver herniation by MRI predicted mortality with%HL AUC = 0.75 with threshold of 21% and LiTR AUC = 0.72 with threshold at 14%.

Recent report has suggested that quantification of liver herniation by two-dimensional US may prove to be another antenatal predictive marker of outcome in CDH. Britto et el, demonstrated strong correlation with US derived LiTR (United States-LiTR) and MRI derive LiTR their respective review of 77 cases of fetal diaphragmatic hernia, US-LiTR and MRI-LiTR ($r = 0.87$; P, 0.001). US-LiTR was comparable to MRI-LiTR in prediction of neonatal mortality [US-LiTR: AUC = 0.78 (95% CI 0.65–0.92), $P < 0.01$; MRI-LiTR: AUC = 0.77 (95%CI 0.63-0.90) $P < 0.01$] and need for ECMO [US-LiTR: AUC 0.72 (95% CI, 0.60–0.85) $P < 0.01$; MRI-LiTR: AUC = 0.73 (95% CI 0.60-0.88) $P < 0.01$].[44] Further studies are needed to validate these findings (Fig. 3). The prognostic value of the o/e LHR when combined with liver position has-been validated to neonatal survival in both left and right isolated CDH (Fig. 4).

Along with mortality, LHR has been shown to be predictive of neonatal morbidity. Russo et al., in their meta-analysis of 38 studies of prenatally assessed diaphragmatic hernia, found that the need for ECMO was predicted by LHR with relative risk (RR) 1.65 (95%CI 1.27- 2.14), o/e LHR standardized mean difference (SMD), −0.70 (95% CI −0.98 to − 0.42), o/e TFLV SMD −1.00 (95%CI −1.52 to −0.48) and%LH RR 3.04 (95% CI 2.23–4.14).[45]

While imaging parameters have proven to be predictive of neonatal mortality due to pulmonary hypoplasia and “need for ECMO”, the studies to date have been less promising in the assessment of the risk for CDH-PH, the other major contributor to perinatal mortality.[45] Various techniques been used to evaluate lung vascularization in an attempt to predict CDH-PH including assessment of Doppler waveform of pulmonary artery and lung perfusion as well as using 3-dimensional power Doppler to evaluate vascular flow indices fractional moving blood volume, and fetal pulmonary vascular reactivity to maternal supplemental oxygen.[46] While these preliminary reports are promising, the predictive value of these and other parameters in predicting CDH-PH has yet to substantiated.

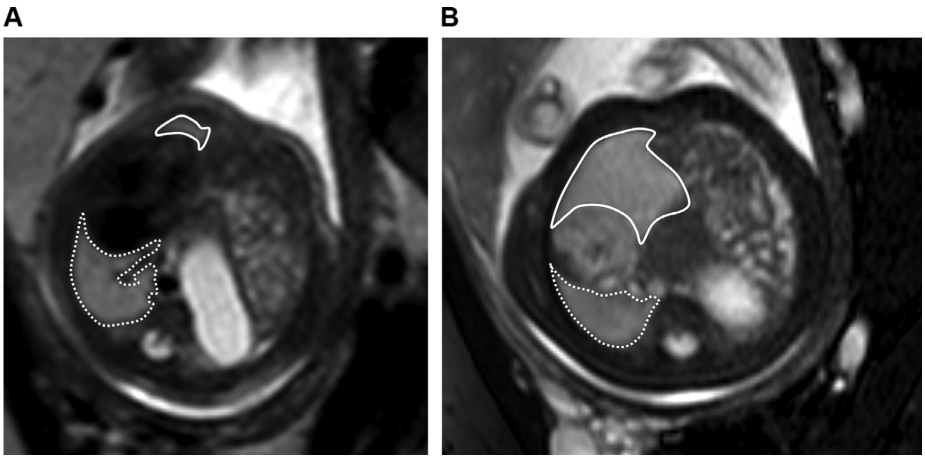

Fig. 2 – Total fetal lung volume (o/e-TFLV) in a fetus with left-sided diaphragmatic hernia. The right lung is outlined by a dotted line and the left is a solid line. (A) Pre-FETO lung areas and (B) Post-FETO lung areas.

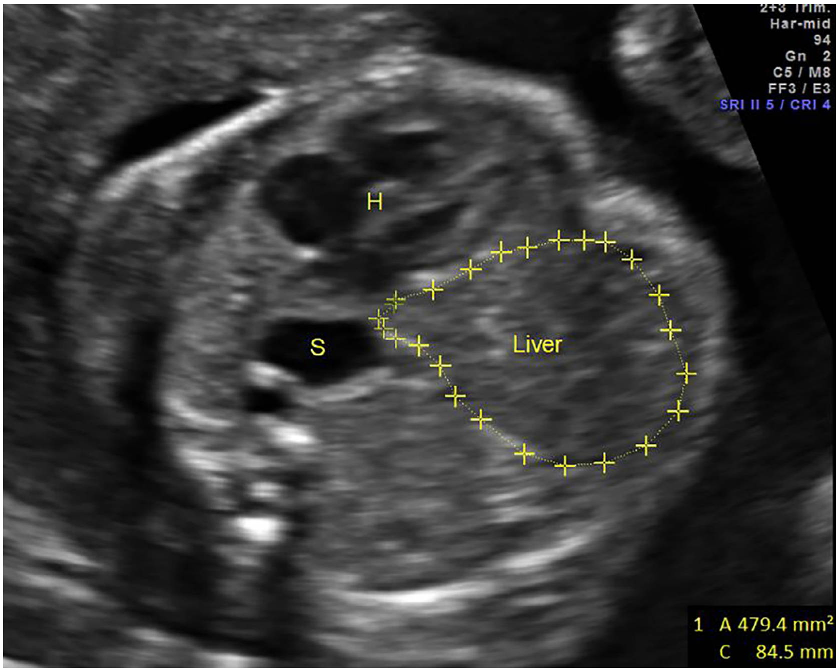

Fig. 3 – Two dimensional ultrasound image of the fetal chest at the level of the four-chambered heart with isolated left sided diaphragmatic hernia with measurement of the intrathoracic liver for the ultrasound derived liver-to-thoracic area ratio (US-LiTR).

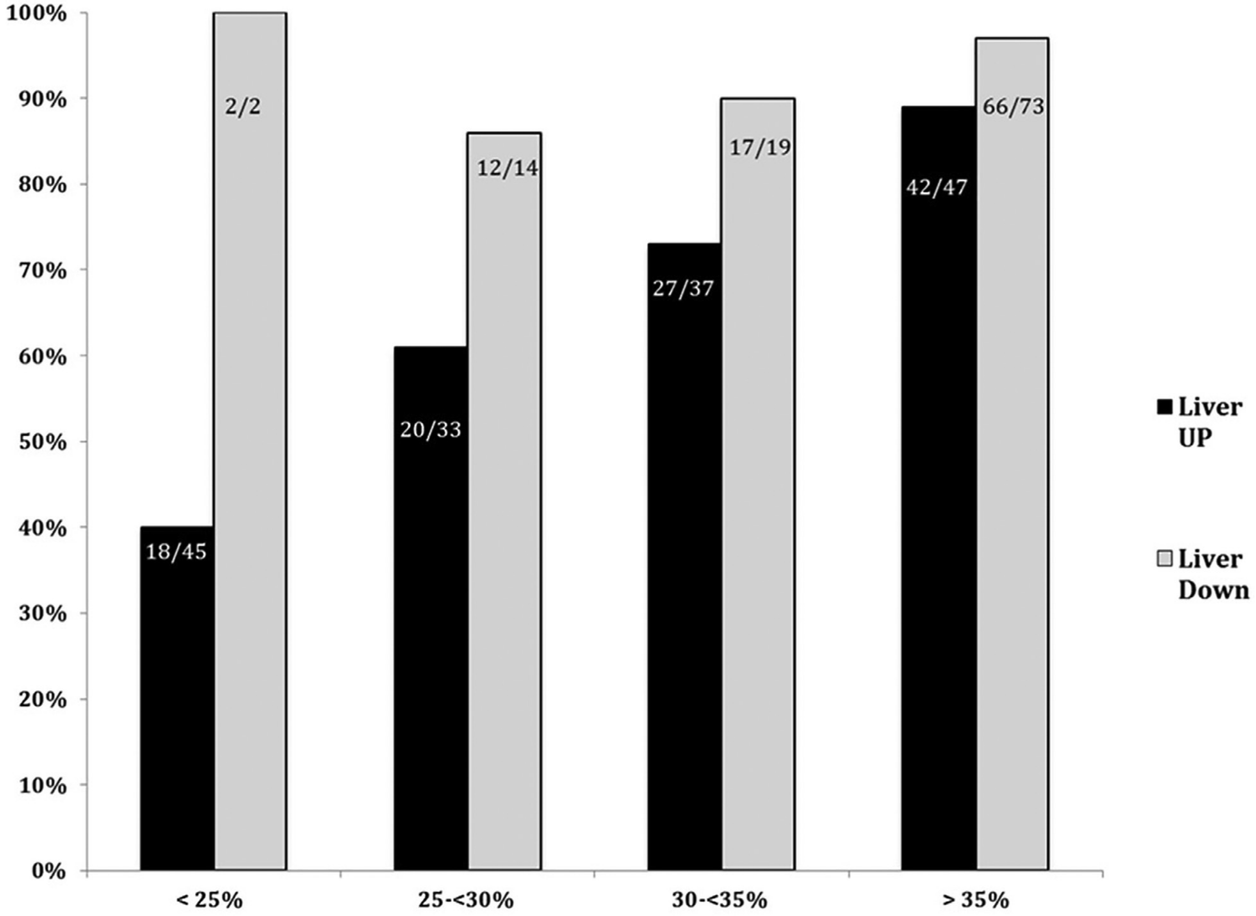

Fig. 4 – Six-month survival rates, left sided congenital diaphragm hernia by LHR O/E cut-offs (5% increments) and liver position (N = 270). Unpublished data from North American Fetal Therapy Network (NAFTNet) FETO Consortium.

Human trials of tracheal occlusion for fetal treatment of severe congenital diaphragmatic hernia

The European-based FETO Task Force developed a technique utilizing a 3.3 mm percutaneous approach with a balloon-type occlusion. Removal of the balloon was facilitated through an ex-utero intrapartum (EXIT) procedure. It was at this time that the results of the United States randomized controlled trial (RCT) of FETO for severe CDH with liver herniation became available.[47] The study found no difference in survival rates between fetal cases that underwent TO with those using the detachable balloon versus those that were randomized to standard postnatal repair, 73 versus 77%. There was a significant difference in the mean gestational age at delivery with the FETO cases delivering at 30.8 weeks versus 37 weeks in the standard care group. Premature rupture of membranes (PROM) prior to 37 weeks played a substantial role in the incidence of prematurity as it occurred in 100% of the FETO cases. The lack of difference in neonatal outcomes was felt to be secondary to the high survival rate of the standard treatment group. The validity of the study came into question based on inclusion criteria, specifically the use of an LHR threshold of <1.4. The majority of the cases enrolled in the trial had LHR values of 1.0 to 1.4, a value consistent with “moderate” pulmonary hypoplasia. Only 9/24 cases, 3 FETO and 6 standard of care, had an LHR that met criteria for “severe” pulmonary hypoplasia based on the results of the European FETO Task Force, specifically an LHR <1.0 at 26−29 weeks (comparable to o/e LHR of 25−27%) with liver herniation). Based on this secondary analysis, the task force concluded that the National Institute of Health trial had not demonstrated lack of benefit for FETO in severe fetal CDH.[48]

The European FETO Task Force report was a prospective series of 210 FETO cases for severe isolated CDH with liver herniation and o/e LHR that was comparable to LHR < 1 when corrected for gestational age. Survival rates in the study group were compared with historical controls managed expectantly from the antenatal registry. The FETO cases were found to have an increased survival rate in both left- and right-sided CDH, from 24 to 49% and 0 to 35%, respectively. PROM was encountered within 3 weeks of the procedure in 16.7% and occurred before 34 weeks in 25%. The median delivery time was 35.3 weeks with 30.9% of patients delivering before 34 weeks.[49] The treatment protocol was to remove the TO after 34 weeks to allow for improved maturation of the lungs and to accelerate type II pneumocyte production. However, 56.2% of cases had an emergent “unplugging” due to complications from PROM, spontaneous labor or the development of hydramnios. Ten cases ended with a neonatal death associated with difficult postnatal removal of the balloon. These cases, unfortunately, delivered outside the FETO centers in programs with limited or absence of expertise in removal of the tracheal balloon. The short term morbidity in these severe isolated CDH cases was found to be similar to that seen in infants with moderate pulmonary hypoplasia who had been managed expectantly, suggesting an improvement in outcome with *in utero* therapy.[50] The main predictors of neonatal outcome in the FETO cases were the o/e LHR before procedure, the gestational age at the time of delivery, and delivery >24 h after removal of the balloon. In a smaller case series from Peralta et al., severe isolated CDH with similar inclusion criteria for severe CDH, analogous to the European FETO Task Force study, exhibited a survival rate of 50% (4/8).[51] The results of another recent RCT from the group in Sao Paulo contradicted that reported by the UCSF group. In this study, severe isolated CDH was defined as LHR <1.0 with liver herniation. Survival rates were significantly higher in the FETO-treated cases compared with the standard postnatal repair cases, 50% (10/20) versus 4.8% (1/21) (relative risk 10.5 [95% CI 1.5−74.7]). Arguably, the neonatal loss rate in the postnatal treated group was appreciably less than was reported by the European FETO Task Force, 20%. This observation would suggest that the neonatal management in the study was not similar to that seen in other programs in the North America and Europe. The findings from the Brazilian group provide further support for the use of FETO in the treatment of severe CDH.

Fetoscopic Endoluminal Tracheal Occlusion procedures

FETO balloon insertion

In the majority of cases, the FETO procedure is performed under local anesthesia. At times a combined spinal−epidural anesthesia may be used, but rarely is general anesthesia required for FETO. Intraoperative US examination is performed to determine placental location and optimal fetal position for trocar insertion. When necessary, external version may be required to achieve better access to the fetal mouth. Once the desired position is obtained, a neuromuscular blocking agent, fentanyl, and atropine are administered to the fetus for immobilization, anesthesia and to minimize risk of fetal bradycardia during the procedure.

Under continuous US guidance, a 3.3 mm cannula with a pyramidal trocar should be inserted percutaneously into the amniotic cavity towards the fetal mouth. Alternatively, entry may be obtained via Seldinger technique. Once secured in position, the trocar is withdrawn and the operative sheath is inserted into the amniotic cavity. This houses the fetoscope, with the occlusive device [a catheter for loading and a detachable latex balloon, Goldball 2; Balt, Paris, FR] and puncture needle in separate channels is inserted. There is a side connector on the sheath to allow amnio−infusion with lactated ringers solution heated to body temperature, for improving visualization and to assist in dilatation of the vocal cords on entry of the trachea.

The endoscope is introduced into the fetal mouth, following the tongue and palate, uvula, epiglottis and vocal cords as landmarks. Once inside the trachea the carina should be identified to position and deliver the balloon between the carina and vocal cords. The balloon should then be inflated with 0.6 mL isotonic fluid and then detached from the catheter. The dimensions of that balloon with inflation are 7 mm diameter by 20 mm in length (Fig. 5a-b). The sheath is removed with amnioinfusion performed if there has been a significant loss of

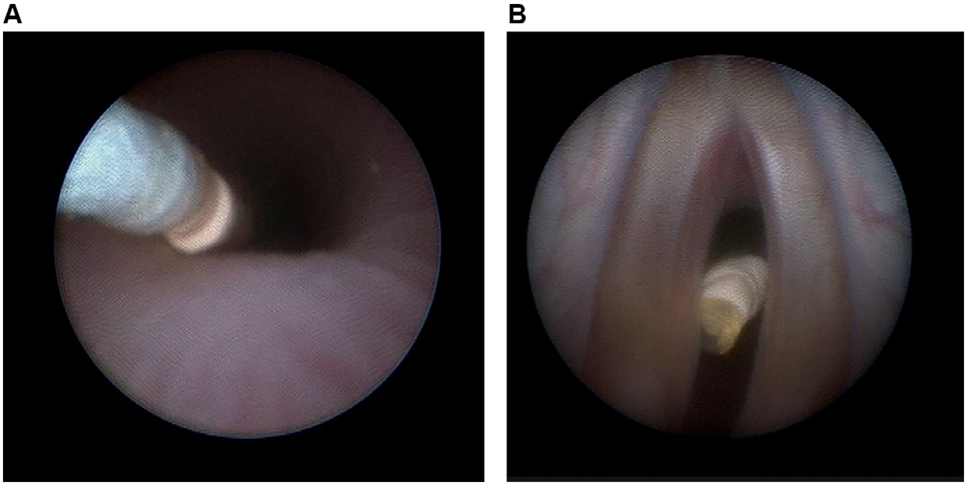

Fig. 5 – Fetal endoluminal tracheal occlusion (FETO) in a fetus with isolated severe diaphragmatic hernia at 28 weeks. (A) Fetoscopic introduction of the occlusive device, catheter and deflated balloon, in trachea. (B) Inflated detached balloon below the vocal cords.

amniotic fluid during the procedure. The median time of FETO is reported to be 10 min (range 3−93 min), depending on operator experience. The failure rate to entering the uterus or trachea, or positioning the balloon, is less than 4%.

FETO balloon removal

Balloon retrieval should be planned at between 34 0/7 and 34 6/7 weeks. The technique may be a fetoscopic retrieval similar to the insertion or US guided puncture, at the discretion of the fetal medicine specialist based on the clinical findings. The US guided puncture technique is performed after fetal medication is given for immobilization and analgesia. The balloon may be removed by tracheoscopy while on placental by-pass at the time of delivery or hopefully, rarely postnatal puncture. In the report by Jimenez et al., of balloon removal in 351 cases elective in 217 cases (72%) and emergent in 85 (28%). In the elective group removal technique was fetoscopic, puncture or on placental circulation in 78.8%, 20.7% and 0.5%, respectively. In the emergent group, fetoscopic, puncture, on placental circulation and postnatal puncture occurred in 35.3%, 21.2%, 38.8% and 4.7% respectively. Nine cases had attempted removal outside of the FETO center. Three of which failed, resulting in neonatal death.[52] The balloon may become dislodged spontaneously due to deflation or increasing tracheal diameter in < 4% of cases prior to planned removal.

Timing of FETO balloon insertion and removal

Arguably in extremely severe CDH, defined as > 30%LH with o/e LHR < 25% (LHR < 0.71), an early intervention should be a consideration, possibly as early as 22 0/7 weeks gestation. However, fetoscopic procedures are associated with an increased risk of iatrogenic prelabor premature rupture of membranes (iPPROM) of upwards of 30%.[53] Specific to FETO, Jani J et al. reported that iPPROM within 3 weeks of insertion of the balloon occurred in 16.7% of the 210 cases.[49,54] While, iPPROM was not associated with outcome, it was associated with gestational delivery. The strongest predictors of outcome were o/e LHR and gestational age of delivery with odds ratios of 1.490 ($P = 0.019$) and 1.024 ($P = 0.007$), respectively. Survival rates in this cohort for deliveries at 25−29% and 30−31 weeks were <10% and 20%, respectively. After 31 weeks' gestation, the survival rates increased to 55−65%. Further, when FETO was performed beyond 30 weeks there was a less profound increase in lung volumes with the occlusion. Based on these data the decision made to set insertion of FETO between 27 0/7 and 29 5/7 weeks' gestation.[55]

Removal at 34 weeks may reduce the amount of lung growth the occlusion might provide and does subject the mother to a second procedure, however, prolonged placement can increase the need for emergent delivery at birth with the associated increased maternal fetal/neonatal risk. Prenatal removal allows for maturation of type 2 pneumocytes and surfactant production and has been associated with improved neonatal outcomes.[56] Therefore, is it recommended at this time that elective balloon removal be performed between 34 0/7 weeks and 34 6/7 weeks.

Preliminary data

Following the European FETO Task Force report, referenced above, there have been a series of single center studies addressing FETO outcomes in "severe" CDH with variable study design (prospective and retrospective), population (left, right and bilateral CDH), and reporting of outcomes. The retrospective report from Ali et al. on 78 CDH infants (43 FETO and 35 infants with contemporary management) found the FETO cases were born earlier (34 vs 38 weeks, $P < 0.001$) and lower mean LHR at referral (0.65 vs 1.24, $P < 0.001$), but not prior to delivery. The FETO group demonstrated longer duration of ventilatory support, supplemental O_2 and hospitalization but there was no difference in the survival to discharge rates between the groups (44.2% vs. 63%, $P = 0.30$).[57]

Persico et al. report their 3 year survival in their retrospective review of 21 cases of severe isolated CDH, defined a o/e LHR < 25% for L-CDH (15) and < 35% in R-CDH (5). There was one bilateral CDH in the group as well. Median gestational age of insertion was 28.1 weeks (range 26.0−31.1) with median gestational age of delivery of 34.7 weeks (range: 31.6−39.0). Emergent balloon removal and iPPROM occurred in 47.6% of cases. Seventeen (47.1%) of isolated unilateral CDH cases survived to 1−3 years.[58]

The retrospective report from Belfort et al. compared neonatal outcomes to historical controls (9) and FETO patients (11) with isolated severe (LHR <1 with liver herniation into the chest) left sided CDH. In 10 of 11 patients, FETO was successfully placed at 27.9 +/- 1.1 weeks. The mean gestational age of delivery in FETO and controls were 35.4 (32.6−39.9) and 38.0 (32.0−39.0) ($P = 0.04$). iPPROM occurred in 27% of FETO cases. There was no difference in the need for ECMO and supplemental oxygen at 6 months between the two groups, however there was significant difference in the survival rates. In the FETO group, 8 of 10 patients survived to 6 months, while in the historic controls survival rates were comparable to what was reported in the Brazilian RCT with only 1 of 9 patients ($P = 0.01$).[59] Clearly, a control group survival of 11% is well below the published literature rate for severe CDH.

Kosinski and Wielgos reported their prospective observation in 28 consecutive case of severe isolated left sided CDH (o/e LHR median 18.9 range 14.1−24.4). FETO insertions ranged from 26.6 to 30.1 weeks (median 27.7) with the median gestational age of delivery 34.7 weeks (range 29.0−38.1). iPPROM occurred in 18% of cases < 34 weeks and 61% < 37 weeks. There were 46.4% neonatal survivors.[60]

A meta-analysis from Al-Maary et al., there were 5 eligible studies including 211 patients with severe isolated CDH (LHR < 1 with liver herniation) where 110 underwent FETO and 101 were controls. FETO favored survival outcome (OR 13.32; 95% CI 5.40−32.87).[61]

While these reports provide additional information and potential support for FETO as an intervention in CDH, the heterogeneous nature of the study designs and reporting should do little to change the conclusion from the 2015 Cochrane review.[62]

TOTAL trial

It is essential that a properly designed RCT be conducted to evaluate the benefit of FETO for CDH. In this regard, the Tracheal Occlusion to Accelerate Lung Growth (www.totaltrial.eu) trial has been initiated by the European Task Force. The trial has two strata for randomization comparing outcome to expectant management, a moderate and severe isolated left sided CDH.

In a moderate pulmonary hypoplasia group, FETO is performed later (30−32 weeks) with the co-primary outcomes, survival until discharge and survival free of oxygen supplementation at 6 months of age. The trial was initiated in August 2010 with a goal to randomized 196 patients. As of May 2, 2019, recruitment was closed with enrollment completed (Clinicaltrials.gov NCT00763737).

In the severe CDH group (Table 1), FETO is performed earlier in gestation at 27 to 29 6/7weeks. The primary outcome is survival until discharge from the neonatal intensive care unit. The study was initiated in November 2011 with a goal of randomizing 116 patients. Participating FETO Centers are attempting to enroll patients in Europe, Japan and North America (Clinicaltrials.gov NCT01240057).

Enrollment has been significantly slower than the moderate arm due to the rare occurrence of isolated cases with o/e

Table 1 – Inclusion and exclusion criteria for the randomized controlled trial comparing FETO to expectant management in severe CDH, the TOTAL trial* (https://totaltrial.eu).

Inclusion criteria	• Patients aged 18 years or more, who are able to consent • Singleton pregnancy • Anatomically and chromosomally normal fetus • Left sided diaphragmatic hernia • Gestation at randomization prior to 29 weeks plus 5 d (so that occlusion is done at the latest on 29 weeks plus 6 d) • Estimated to have severe PH, defined prenatally as: o/e LHR < 25%, irrespective of the liver position • Acceptance of randomization and the consequences for the further management during pregnancy and thereafter. • The patients must undertake the responsibility for either remaining close to, or at the FETO center, or being able to travel swiftly and within acceptable time interval to the FETO center until the balloon is removed. • Intended postnatal treatment center must subscribe to suggested guidelines for “standardized postnatal treatment”. • Provide written consent to participate in this RCT
Exclusion criteria	• Maternal contraindication to fetoscopic surgery or severe medical condition in pregnancy that make fetal intervention risk full • Technical limitations precluding fetoscopic surgery, such as severe maternal obesity, uterine fibroids or potentially others, not anticipated at the time of writing this protocol. • Preterm labor, cervix shortened (<15 mm at randomization) or uterine anomaly strongly predisposing to preterm labor, placenta previa • Patient age less than 18 years • Psychosocial ineligibility, precluding consent • Diaphragmatic hernia: right-sided or bilateral, major anomalies, isolated left-sided outside the o/e LHR limits for the inclusion criteria • Patient refusing randomization or to comply with return to FETO center during the time period the airways are occluded or for elective removal of the balloon • Diaphragmatic hernia: right−sided or bilateral, * major associated anomalies, isolated left−sided outside the o/e LHR limits for the inclusion criteria • Patient refusing randomization or to comply with return to FETO center during the time period the airways are occluded or for elective removal of the balloon

* [ClinicalTrials.gov Identifier: NCT01240057].

LHR <25% and treatment of patients outside the trial. As of June 28, 2019, only 76 patients have been enrolled in this arm of the trail. The North American Fetal Therapy Network (NAFTNet) FETO Consortium presently has 10 potential FETO centers who could increase the rate of enrollment once all become eligible to participate. As of this writing, two of the programs are actively participating, one is eligible awaiting FDA approval, four are completing the centers feasibility study and three eligible centers have “lost equipoise” and are offering FETO for severe isolated CDH outside of the trial.

Trial limitations

Subjects with severe cases who are unaware of the trial or have declined randomization continue to receive fetal intervention at non-FETO sites who have approval to use the balloon but no longer have equipoise. This has resulted in a subsequently delayed recruitment and potentially biased the study. The extent to which non−FETO centers will carry out FETO in patients that are eligible for the herein proposed RCT cannot be estimated. Maximum efforts from medical societies, regulatory agencies and third party payers should be applied to reduce the number of such centers. At present, there are centers that have not committed to joining the severe arm of TOTAL Trial. If these centers continue to offer FETO outside of the trial, this would be a “backdoor” to the trial, delaying completion of the trial and introducing a potential source of error in the results of the TOTAL Trail.

Participating FETO centers must meet strict criteria based on sufficient number of postnatal managed CDH cases and fetoscopic procedures performed annually and must undergo rigorous training for FETO before participation. Neonatal care is moving toward more standardized management practices. The study is an international multicenter trial with participating FETO centers in Europe and North America. Qualified centers in the United States have filed applications to the Food and Drug Administration for use of the fetoscopic instruments required to participate in the trial in early 2011. As of the date of this publication, the investigation device exemption has yet to be approved. While it is recognized that these regulatory constraints continue to delay advancements in the field of fetal medicine in the United States, it would seem unethical to continue to offer this intervention outside the bounds of a RCT.

Conclusion

FETO holds the promise of an effective antenatal therapy for the treatment of fetal CDH. As such the studies reported to date have demonstrated the feasibility of this intervention. However, there is still insufficient comparative evidence to recommend *in utero* fetal intervention for CDH at this time as part of routine clinical care. Future studies are needed to examine the benefits of subgroups for FETO, moderate and severe CDH cases. These research endeavors should include a standardization of the procedures (including prenatal intervention and postnatal care), inclusion criteria and long-term childhood follow-up.[62] FETO for the treatment of antentally diagnosed CDH should only be conducted under investigative protocols.

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None.

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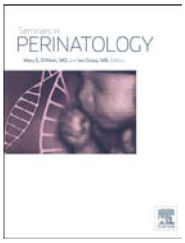

Prediction tools in congenital diaphragmatic hernia

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ABSTRACT

Because congenital diaphragmatic hernia (CDH) is characterized by a spectrum of severity, risk stratification is an essential component of care. In both the prenatal and postnatal periods, accurate prediction of outcomes may inform clinical decision-making, care planning, and resource allocation. This review examines the history and utility of the most well-established risk prediction tools currently available, and provides recommendations for their optimal use in the management of CDH patients.

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Introduction

Congenital diaphragmatic hernia (CDH) has long been recognized as a disease characterized by a wide spectrum of severity.[1] Greater or lesser degrees of lung hypoplasia and pulmonary hypertension, the contribution of additional anomalies, and the particular anatomy of the diaphragm defect, along with overall cardiopulmonary function, all combine to result in a highly variable clinical phenotype. In many cases, this phenotype will be predictable based on prenatal imaging and patients may be appropriately risk-stratified well before birth. However, at least one third of patients are not prenatally diagnosed, and more importantly, patient risks are not static. Gestational changes, the transition from fetal circulation, and iatrogenic factors after birth may greatly affect the clinical status of the patient from moment to moment. "Risk", as such, is potentially highly mutable, and repeated estimates generate an overall risk trend, which is likely to be far more accurate in predicting an outcome than any single-stage measure of severity.

Unsurprisingly, given the heterogeneous CDH population, innumerable variables and tools have been described over the past 40 years that predict the risk of certain outcomes and may be applied at various time points. Few instruments have been developed using rigorous methodology, very few such instruments have been validated using secondary cohorts, and fewer still have been widely adopted. Some are not designed for prospective clinical application but rather for retrospective data analysis. Many predictive variables to one extent or another are surrogate measures of functional gas exchange, as the ability for the cardiopulmonary system to provide adequate alveolar-arterial O_2 and CO_2 transfer and systemic oxygen delivery will ultimately determine the likelihood of adverse outcomes.

The purpose of this review is to provide an overview of the history and current status of risk prediction in CDH, in order to equip practitioners with the necessary tools to anticipate care needs, inform decision-making, allocate resources appropriately, and optimize outcomes in this complex disease.

Why do we need prediction tools in CDH?

Broadly speaking, the key purposes of risk stratification in complex patients are to identify groups who might benefit from a particular intervention and to enable risk-adjusted analysis of outcomes, costs, and management approaches.[2,3] Because CDH is characterized by a spectrum of severity, risk

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stratification is essential for family counseling, resource allocation, establishment of care limits, and, if a prenatal diagnosis has been made, delivery planning, termination, or fetal intervention. Postnatally, essential management decisions such as timing of surgical repair and the use of invasive and expensive treatment modalities are fundamentally informed by knowledge of the patient's risk profile. Evidence is emerging that *severity-specific management* results in not only higher survival for high-risk CDH patients, but also potentially reduces complications and cost of care in lower-risk patients by avoidance of intensive therapies.[4]

The CDH risk prediction literature is surprisingly rich. From this large body of work, it has become apparent that there are distinct risk strata of CDH patients that will likely benefit from targeted care pathways. For example, high-risk patients identified prenatally may be targets for fetal intervention, and those identified postnatally may benefit from early anticipated transition to extracorporeal membrane oxygenation (ECMO) or early repair on ECMO[5]. Unfortunately, there is as of yet no universally accepted method for risk-stratification and certainly no agreed-upon set of risk-specific management guidelines. In the following sections, we have made suggestions regarding clinical management that may be derived from application of the described prediction tools.

Prenatal prediction tools (Table 1)

There is a long history of prenatal risk prediction in CDH, which really began with the advent of the use of US to identify the lesion in the fetus in the early 1980′s.[6] Prenatal diagnosis of CDH is, in itself, associated with worse survival, because the largest defects are more likely to be detected.[7] The desire to fix CDH prenatally in order to improve survival led the team under Harrison at UCSF to search for features of the condition that predict the worst outcomes, such that fetal intervention may be considered in those patients. Over time, our understanding of the anatomy has vastly improved along with the accuracy of prenatal imaging.

Lung-to-head ratio (LHR)

The LHR, which is the ratio of the contralateral lung area (mm^2, obtained at level of the 4-chamber heart view) to the head circumference (mm), was developed for counseling and delivery planning purposes, and also to select appropriate patients for fetal intervention early in gestation. It estimates the likelihood of pulmonary hypoplasia and hence survival, with a measurement of <1.0 portending a poor prognosis, and >1.4 suggesting virtually 100% survival. Excellent papers are available that summarize the literature and describe in detail the optimal techniques for measuring LHR.[8–10]

Prior to the first description of LHR from UCSF in 1996,[11] antenatal prognostication for CDH patients was inconsistent and derived from such ultrasound (US)-detected findings as liver and stomach herniation into the thorax, polyhydramnios, early detection of the defect (<25 weeks GA), heart morphology, and lung area to thorax area ratio.[12,13] The LHR was the first widely adopted method of adjusting a prenatal measure of lung size using an unaffected part of the fetus's own anatomy, in this case the head circumference, as a normalizing ratio. This was done "to minimize lung size differences owing to gestational age", but unfortunately in practice the LHR changes markedly as gestation progresses since the lung grows more than four times faster than the head.[10] This limits its reproducibility and predictive ability, as does marked interobserver variation in calculating LHR, variable ultrasonographer and center experience, and the fact that there are three different described ways to measure lung area.[13] Additionally, LHR was initially designed only for left CDH using the right lung area, and published ranges for outcomes prediction are largely based on LHR data calculated at 24–26 weeks' GA, which renders LHR measurements outside this range less accurate for prediction.[14] For these reasons, simple LHR has fallen out of favor, particularly after publication of data showing poor prognostic accuracy.[8,15]

Observed-to-expected (O/E) LHR

In 2005, publication of a valuable report established reference LHR values in 650 normal fetuses throughout gestation.[16] These data enabled the development of the O/E LHR,[17] which works for right and left CDH and may be applied to any age fetus, as the O/E LHR value does not significantly change as GA increases (Fig. 1). Reports have consistently shown that survival is less than 50% with an O/E LHR of less than 25%, and exceeds 80% with an O/E LHR of greater than 40%[17–19] (Fig. 2). While still hampered to some degree by interobserver variability, the O/E LHR has become widely adopted by most centers as the primary tool to assess risk prenatally in CDH. Of the three well-described methods of measuring lung area, the tracing method appears to yield the best predictive accuracy.[21,22]

O/E total fetal lung volume (TFLV) and absolute fetal lung volume (FLV)

Over the past decade, there has been a marked increase in the use of fetal magnetic resonance imaging (MRI) to predict survival, by measuring lung volume to estimate the degree of pulmonary hypoplasia. A multicenter European group generated reference values for TFLV using 336 normal fetuses[23] and then demonstrated that O/E TFLV is significantly associated with survival in a small CDH case series.[24] The same group then prospectively assessed O/E TFLV in 77 CDH patients, and found that an O/E ratio of <25% was associated with a significant decrease in survival.[25] This technique employed fast spin-echo T2-weighted lung MRI. Multiple groups have since refined the MRI technique and shown that the O/E TFLV is an accurate and reproducible risk stratification tool with good discrimination for not only survival (the pooled C statistic was 0.8 in a 2017 meta-analysis[22]), but also ECMO use and pulmonary morbidity.[3,26–28] Measured absolute FLV even without the use of the O/E ratio is by itself predictive of survival and use of ECMO.[29–31] O/E TFLV may have better discrimination[22] and, like O/E LHR, provides consistent values despite GA and therefore has greater generalizability, reproducibility, and ease of interpretation.

Table 1 – Validated prenatal prediction tools

Predictor/score (publication date)	Outcome(s)	Variable(s)	Timing	Derivation population/study (N)	Select validation studies (N)	Model/formula/method	Best C statistic or other measure of performance	Limitations or comment
Lung-to-head ratio (LHR, Metkus 1996)	survival	area of right lung/ head circumference, using US	24–26 weeks	single center (55)	Lipshutz 1997 (15) Yang 2007 (107) Keller 2003 (56)	area of right lung (mm^2)/head circumference (mm)	C 0.73 (Keller 2003); C 0.7 (Bebbington 2014)	Varies with GA; multiple measurement methods used; operator dependent; not valid for right CDH
O/E LHR (Jani 2007)	survival	ratio of area of contralateral lung/ head circumference to expected mean LHR for GA, using US	independent of GA	multicenter (354)	Alfaraj 2011 (72) Madenci 2013 (37) Bebbington 2014 (85) Werner 2016 (55) Snoek 2017 (122) Senat 2018 (223)	LHR/mean expected LHR Tracing method: Expected LHR, left CDH: $-2.3271 + (0.27*GA)-(0.0032*GA^2)$ Expected LHR, right CDH: $-1.4994 + (0.1778*GA)-(0.0021*GA^2)$	C 0.81 (Madenci 2013); C 0.80 (Alfaraj 2011); C 0.79 at large centers (Senat 2018); C 0.78 (Oluyomi-Obi 2017)	Predictive accuracy depends on operator/center experience; tracing method to measure lung area is probably ideal; also predicts pulmonary hypertension and morbidity
O/E total fetal lung volume (TFLV) or relative FLV (Mahieu-Caputo 2001, Gorincour 2005)	survival	ratio of FLV to expected FLV, using MRI	20–33 weeks	multicenter (77)	Cannie 2008 (40) Ruano 2014 (80) Akinkuotu 2016 (176)	FLV at the time of MRI / expected FLV (EFLV) at same GA given by the normative curve $EFLV = 0.0033\ g^{2.86}$, where g=GA	C 0.85 (Cannie 2008); C 0.8 (Oluyomi-Obi 2017); C 0.78 (Ruano 2014); C 0.71 (Gorincour 2005)	
Absolute FLV (Neff 2007, Busing 2008)	survival	FLV assessed with MR planimetry	32–34 weeks	single center (65)	Kilian 2009 (36) Lee 2011 (44)	A hand-tracing drawing tool outlined lung boundaries on consecutive images of entire thorax; computer algorithm calculated the area in mm^2; area multiplied by slice thickness to obtain entire lung volume	C 0.74 (Lee 2011)	
% predicted lung volume (PPLV, Barnewolt 2007)	survival	Measured lung volume / predicted lung volume, using MRI	median 22 weeks	single center (14)	Madenci 2013 (37) Ruano 2014 (80)	Predicted lung volume (PLV) = total thoracic volume - mediastinal volume PPLV = total lung volume / PLV	C 0.82 (Madenci 2013) C 0.74 (Ruano 2014)	
Liver herniation (Harrison 1990)	survival	presence of liver above diaphragm / in thorax on prenatal US or MRI	not specified	single center (45)	Albanese 1998 (48) Kitano 2005 (30) Hedrick 2007 (89) Mullassery 2010 (407)	Various criteria; based on position of liver parenchyma and/or vasculature including hepatic and portal vessels (Mullassery 2010)	sensitivity 73%, specificity 54% (Mullassery 2010)	Not applicable for right CDH; variable definition of and markers for liver herniation; most useful in concert with primary prediction tool; also

Table 1 (*continued*)

Predictor/score (publication date)	Outcome(s)	Variable(s)	Timing	Derivation population/study (N)	Select validation studies (N)	Model/formula/method	Best C statistic or other measure of performance	Limitations or comment
								predictive of ECMO use (Russo 2017)
Herniated liver volume to thoracic volume ratio (LiTR, Cannie 2008)	survival	ratio of liver volume herniated into thoracic cavity to volume of thoracic cavity, using MRI	median 30 weeks	single center (40)	Worley 2009 (15) Ruano 2014 (80)	Intrathoracic liver volume / thoracic cavity volume Liver considered thoracic when above a line drawn from lower tip of xyphoid process to corresponding vertebral body at same level	C 0.91 (Worley 2009) C 0.88 (Cannie 2008) C 0.72 (Ruano 2014)	
% liver herniation (%LH, Lazar 2012)	survival	ratio of herniated liver volume to total fetal liver volume, using MRI	not specified	single center (53)	Ruano 2014 (80) Akinkuotu 2016 (176)	Herniated liver volume / total fetal liver volume Herniated liver defined on coronal plane imaging as liver located above level of contralateral diaphragm; volumes measured by manual tracing and summation of traced areas multiplied by slice thickness	C 0.75 (Ruano 2014); C 0.74 (Lazar 2012)	
Stomach herniation (Cordier 2015)	survival	presence of stomach above diaphragm / in thorax using prenatal US	Not specified	single center (114)	Gentili 2015 (77) Basta 2016 (90)	Grade 1, stomach not visualized; Grade 2, stomach visualized anteriorly, next to apex of heart, with no structure in between stomach and sternum; Grade 3, stomach visualized along from apex of heart and abdominal structures anteriorly; or Grade 4, Grade 3 with stomach posterior to level of atrioventricular heart valves	C 0.83 (Basta 2016); C 0.72 (Gentili 2015)	Not applicable for right CDH; useful as marker for assessing liver position

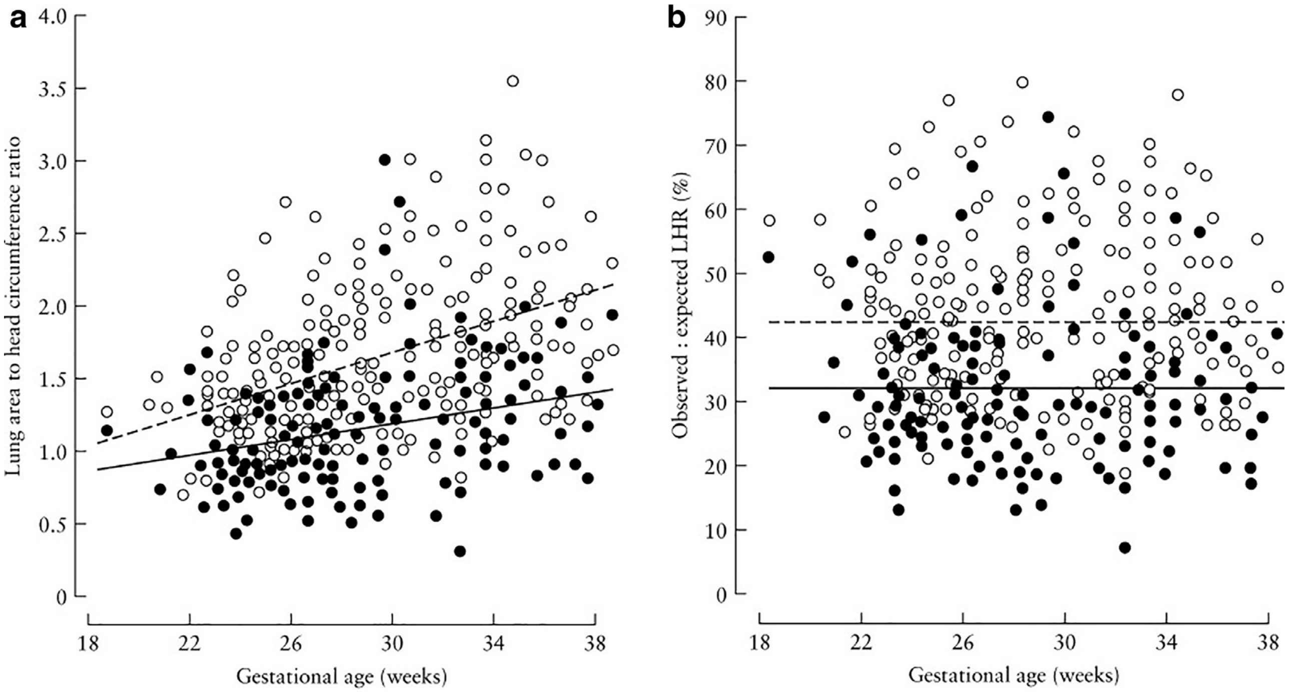


Fig. 1 – Relationship between right lung area to head circumference ratio (LHR) (a) and observed to expected LHR (b) with gestational age in normal fetuses.[17] Lines represent mean and 95% CI. Copyright © 2007 ISUOG. Published by John Wiley & Sons, Ltd. Reproduced with permission.

The O/E TFLV by MRI is generally believed to be superior to O/E LHR in predictive accuracy, although a meta-analysis has demonstrated that if the tracing method is used for the LHR calculation, the two techniques have equivalent discrimination.[22] In that study, both measurements were found to be associated with decreased survival at the 25% O/E threshold.

3D US is also used to measure lung volume in CDH. However, this modality has not been widely adopted as the ipsilateral lung is not reliably visualized with US. MRI remains the modality of choice when assessing TFLV.[32]

Liver herniation (LH), liver volume to thoracic volume ratio (LiTR), and% liver herniation (%LH)

For decades, the presence of liver in the chest (LH or “liver up”) has been recognized as a feature of severe CDH. In 1990, Harrison et al. reported a 100% mortality rate in fetuses with this clinical feature.[33] LH, which is present in about half of left CDH cases,[34] tends to occur in large defects and is associated with a greater degree of lung hypoplasia than in CDH patients without LH. Along with O/E LHR, LH is a long-

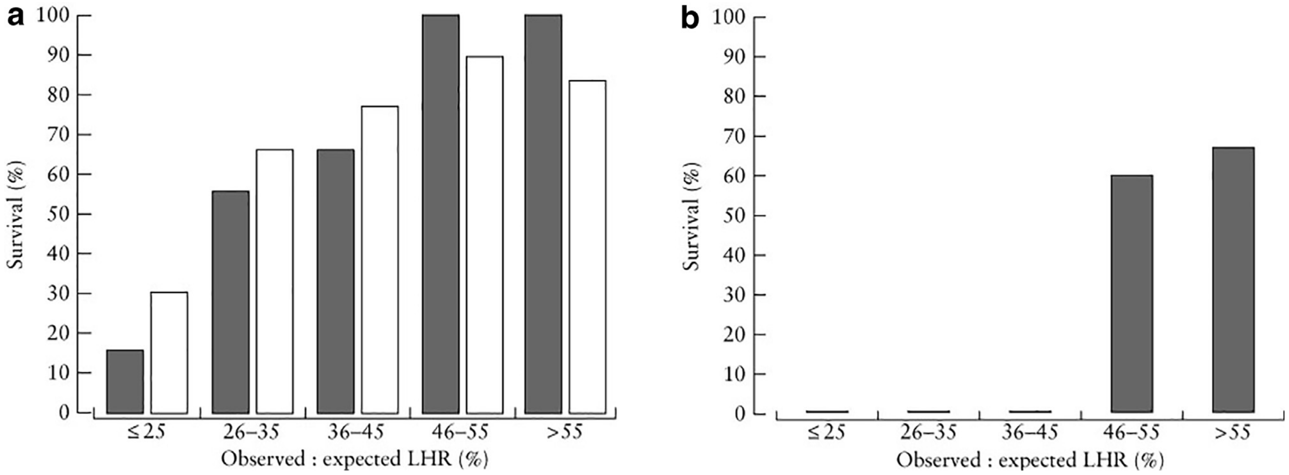


Fig. 2 – Survival rate according to the fetal observed to expected lung area to head circumference ratio (LHR) in fetuses with isolated left-sided (a) and right-sided (b) diaphragmatic hernia. The filled bars represent fetuses with intrathoracic herniation of the liver and the open bars represent those without herniation.[17] Copyright © 2007 ISUOG. Published by John Wiley & Sons, Ltd. Reproduced with permission.

standing marker of severity that has been used to select high-risk patients for fetal intervention.[20,35] LH is also predictive of needing a subsequent patch repair to close the defect.[36]

Imaging has become increasingly sensitive; US and, especially, MRI are able to determine when even a small proportion of the liver herniates into the chest. A meta-analysis found that while LH predicts mortality with a sensitivity of 73% and specificity of 54%, the prognostic utility of this binary variable is limited largely because of the inconsistent definition of “liver herniation” amongst studies.[37] No studies have provided a C statistic to assess the discrimination of LH for survival (Table 1), though another meta-analysis, in addition to finding LH to be significantly associated with decreased survival, found that LH may improve LHR discrimination if the two assessments are combined.[38]

Because of the binary nature of LH, the relative proportion of herniated liver has gained popularity and is a highly accurate prognostic tool, matching or exceeding the discrimination of O/E LHR in some reports.[27] Proportion of herniated liver was first described as the ratio of herniated liver volume to total thoracic volume (LiTR), which was shown in several studies to correlate well with survival.[27,39,40] Alternatively, the volume of herniated liver expressed as a ratio to total liver volume (%LH) is well-studied, and may be easier to measure and less variable than total thoracic volume.[27,41] In addition to good survival prediction, with a threshold of >20% of liver herniated associated with high mortality,[4]%LH has also been found to be a strong independent predictor of long-term adverse pulmonary sequelae.[28]

Stomach herniation

Postnatally, Goodfellow and Burge et al. identified that intrathoracic stomach herniation is significantly associated with worse survival compared to an intraabdominal position, and surmised that antenatal observation of this feature could predict mortality.[42,43] Although frequently mentioned as a marker for severity, it has never gained prominence as a quantifiable variable, though some studies have shown significant correlation with adverse outcomes; reproducibility and ease of measurement are advantages.[44,45] Stomach herniation may be most useful as a marker of liver position, obtained at the level of the four-chamber heart view.[10]

Other prenatal prediction tools

Prenatal imaging quality, precision, and experience continue to improve year by year, and other observable fetal characteristics may provide equivalent or better predictive capability as technology evolves. Studies have identified such predictive fetal variables as pulmonary artery diameter,[46] FLV to body weight ratio,[47,48] and the McGoon index [(right pulmonary artery diameter + left pulmonary artery diameter)/aorta diameter],[49] among many additional tools and combinations of tools that have not been validated. As MRI technology and software become more advanced, it may become possible to accurately construct a three-dimensional image of the fetal diaphragm, which could correlate with subsequent clinical severity and greatly facilitate operative planning.[50]

Postnatal prediction tools (Table 2)

Predicting the risk of adverse outcomes remains important for infants after birth, and prenatal prediction tools are often insufficient for risk prediction postnatally. Many infants are born without a prenatal diagnosis or have had limited imaging prenatally. Infants may be born with new risks such as low birth weight that were not relevant as a fetus but play a major role in determining the outcome after birth. Some CDH newborns with a prenatal diagnosis will have better or worse pulmonary function at birth than expected, and, even when optimally performed, prenatal imaging is not always predictive of outcome.[51] A survival probability should be recalculated in all newborns to solidify or modify any prenatal risk stratification and to help guide care. Many good prediction models exist, and most have been derived from or validated with large registries, rather than from single centers, as is often the case with prenatal prediction tools.

CDH study group predictive survival (CDHSG-PS)

Based on the infant’s response to resuscitation, the first few hours of life will reveal the severity of pulmonary hypoplasia that is present in the newborn CDH patient. Indeed, one of the first popular postnatal prediction tools, developed using the CDHSG registry in 2001, is applied immediately after birth using the 5-minute Apgar score and birth weight to generate a survival probability.[52] CDHSG-PS remains accurate for survival prediction nearly two decades later, despite many touted advances in neonatal resuscitation and management,[53−55] though some centers have not shown a consistent association with survival[56] and the model has suboptimal calibration.[57] Clearly, quality of resuscitation and patient management will affect prediction accuracy center-to-center. The tool was not designed for use in determining individual patient outcomes, and Apgar scores in resuscitated newborns must be interpreted with caution because elements of the score may be affected by resuscitation.[58] However, this simple calculator provides at least a rough estimate of risk postnatally and may be accessed online at mobile.nicutools.org.

Blood gas variables

Blood gas measures provide a dynamic measure of risk that reflects both the physiology of the infant and the care being provided. Poor gas exchange and a failure to improve markers of oxygenation and ventilation is a poor prognostic sign in CDH newborns,[59] and blood gas values have long been used to accurately predict outcome and serve as markers for transitions in care.[60] Many highly accurate risk stratification tools have been reported that incorporate arterial or capillary blood gas values from the first hours of life.[54,56,59,61−65] The most accurate and commonly reported variable in these tools is the partial pressure of CO_2 (PCO_2), which is predictive of survival whether the first blood gas,[54,61] highest value,[64] or lowest value[65] during the postnatal period is used. The inability to clear CO_2 is a very good measure of overall cardiopulmonary function in the CDH patient and, unlike oxygenation,

Table 2 – Validated postnatal prediction tools

Predictor/score (publication date)	Outcome(s)	Variable(s)	Timing	Derivation population/study (N)	Select validation studies (N)	Model/formula/method	Best C statistic or other measure of performance	Limitations or comment
CDHSG predicted survival (2001)	survival	BW, 5 min Apgar	immediate postnatal	CDHSG (1054)	Skarsgard 2005 (88) Baird 2008 (94) Gentili 2015 (77) Sekhon 2019 (203)	probability of survival = $1 - 1/(1 + e^{-x})$, where: $-x = -5.024 + 0.9165(BW) + 0.4512(Apgar5)$ BW= birth weight in kg Apgar5 = 5 min Apgar	C 0.87 (Gentili 2015); C 0.85 (Baird 2008); C 0.83 (Skarsgard 2005); C 0.77 (Sekhon 2019)	Poor calibration Online calculator available at mobile.nicutools.org
CDHSG defect size (2013)	survival	Size of CDH defect	At operation	CDHSG (1638)	Werner 2016 (55)	"A" defects are entirely surrounded by muscle; "B" defects have a small (<50%) and "C" defects a large (>50%) portion of the chest wall devoid of diaphragm tissue; "D" patients have complete or near complete absence of the diaphragm	C 0.9 (Werner 2016)	Can only be measured at operation or autopsy; useful for outcomes comparison between centers and cohorts
Wilford Hall/Santa Rosa clinical prediction formula ($WHSR_{PF}$, Schultz 2007)	survival	Highest PO_2 and CO_2 during day 1	24 h of life	Single center (88) & CDHSG (849)	Hoffman 2011 (62) Park 2013 (114) Gentili 2015 (77) Sekhon 2019 (203)	score = PO_2[max] - PCO_2[max]; highest values obtained during day 1	C 0.84 (Gentili 2015); C 0.8 (Sekhon 2019); C 0.8 (Park 2013); C 0.79 (Schultz 2007); C 0.71 (Hoffman 2011)	
Brindle score (2014)	mortality	BW, 5 min Apgar, pulmonary hypertension (ECHO), cardiac/chromosomal anomaly	early postnatal	CDHSG (2202)	Akinkuotu 2016 (176) Bent 2018 (705) Closhe 2018 (162) Sekhon 2019 (203)	1(low birth weight) + 1 (low 5 min Apgar) + 2 (missing 5 min Apgar) + 2(severe pulmonary HTN) + 2 (major cardiac anomaly) +1(chromosomal anomaly) = total score (8 max)	C 0.81 (Brindle 2014); C 0.8 (Clohse 2018); C 0.74 (Bent 2018); C 0.74 (Sekhon 2019)	Use of a missing variable as a predictor may affect generalizability
SNAP-II (Skarsgard 2005)	survival	mean blood pressure, lowest temperature, PO2/FIO2 (%) ratio, lowest serum pH, presence of seizure activity, and urine output	12–24 h of life		Baird 2008 (94) Coleman 2013 (47) Gentili 2015 (77) Snoek 2016 (171)	worst score within the first 12 h of life for each variable as described by Richardson 2001	C 0.88 (Snoek 2016); C 0.81 (Skarsgard 2005), with GA; C 0.79 (Gentili 2015); C 0.79 (Baird 2008); C 0.77 (Coleman 2013);	The only predictor with level 2 evidence (Snoek 2016); addition of GA improves discrimination; cumbersome to calculate; variables not measured in CDHSG registry

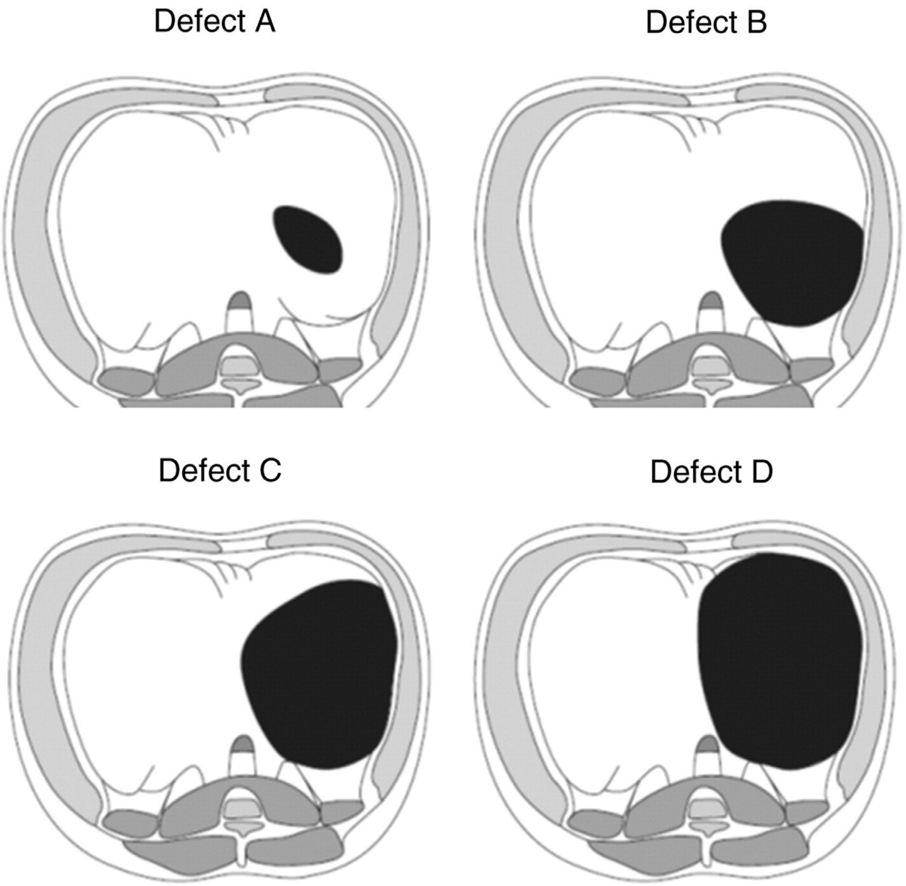


Fig. 3 – CDHSG defect size diagram, drawn with the diaphragm (defect) on the patient's left looking up from the abdomen towards the chest. "Defect A": smallest defect, usually "intramuscular" defect with >90% of the hemi-diaphragm present; this defect involves <10% of the circumference of the chest wall; "Defect B": 50–75% hemi-diaphragm present; this defect involves <50% of the chest wall; "Defect C": <50% hemi-diaphragm present; this defect involves >50% of the chest wall; "Defect D": largest defect (previously known as "agenesis"); complete or near complete absence of the diaphragm with <10% hemi-diaphragm present; this defect involves >90% of the chest wall. "D" defects should all require a patch (or muscle flap) for repair. Reprinted from Journal of Pediatric Surgery, 48:12, Kally KP et al., Standardized reporting for congenital diaphragmatic hernia – An international consensus, Copyright © 2013, with permission from Elsevier.

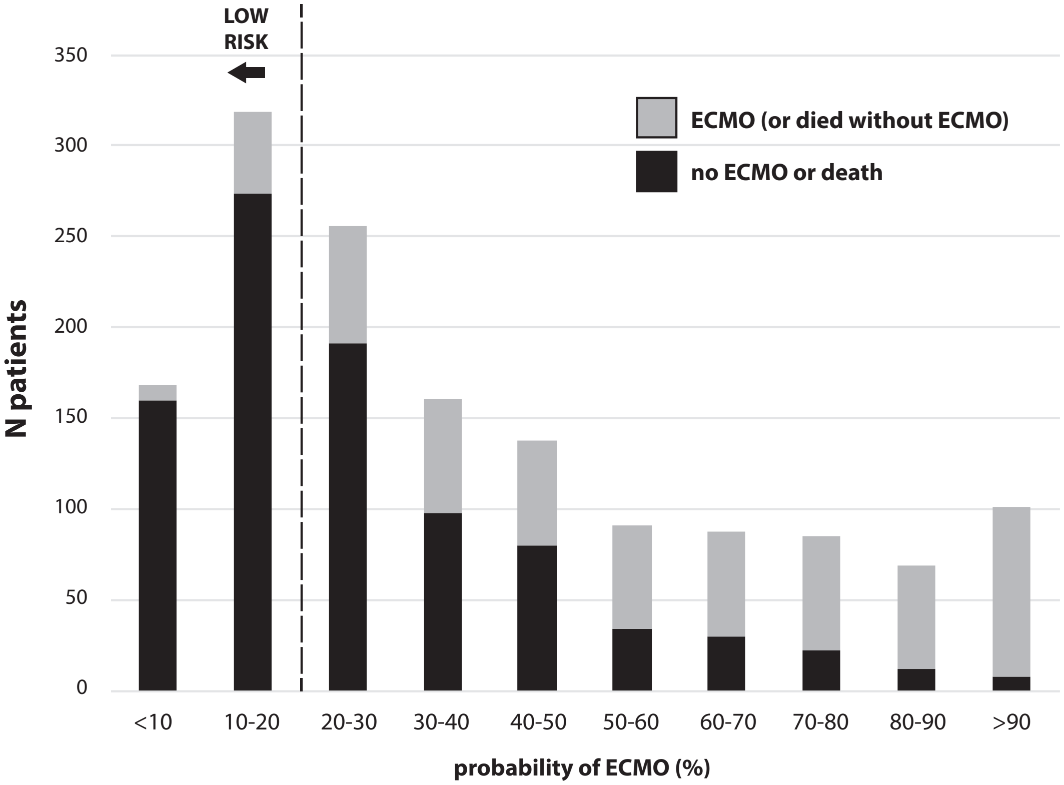
will not respond as readily to resuscitative maneuvers, therefore likely improving the discrimination of PCO_2 over PaO_2 in survival prediction. However, PaO_2 and pH are both strong predictors of outcome as well,[54] as is the best preductal O_2 saturation.[66] There is a reported threshold of 70 mmHg for lowest pre-ECMO PCO_2 beyond which 100% mortality has been observed, and the CDHSG registry cutoff value for "high risk" is 55–60 mmHg for lowest PCO_2 during the first 24 h of life.[65]

The Wilford Hall/Santa Rosa clinical prediction formula ($WHSR_{PF}$) uses the difference between the highest PaO_2 and the highest PCO_2 to generate a score that predicts survival, with a value greater than 0 portending a good prognosis.[63] This score has been repeatedly validated in the literature[53,56,62] but is not used widely, perhaps because it was derived using a small cohort and has not consistently performed well across studies.[67]

As time elapses after birth, provided interventions such as choice of ventilator will greatly affect the physiology of the patient and hence blood gas values will increasingly reflect response to treatment, becoming less accurate in terms of their true representation of underlying physiology. Predictive accuracy, in fact, decreases within hours of birth.[54,61] Hence, the initial blood gas or highest or lowest values should be used for risk stratification purposes.

CDHSG defect size

As a marker for degree of pulmonary hypoplasia, thoracic compression, and/or severity of developmental insult, the size of the CDH defect is significantly correlated with survival, need for patch repair, and other adverse outcomes.[68] Beginning in 2007, member centers of the CDHSG registry began recording defect size during surgical repair of the CDH (Fig. 3). A major cardiac anomaly upstages the defect. The data on 1638 patients were reported in 2013,[69] and the staging system was since independently validated with a C statistic of 0.9 for survival.[70] This staging system was designed to serve as a standard for reporting and not as a predictor for individual patients. Currently, defect size staging is primarily used for post hoc outcomes research and inter-center comparison. Its role in guiding care is hampered by the fact that it cannot



$$\text{Probability of ECMO} = \frac{e^{(-2.0191) + (-0.1407*A) + (-0.1939*B) + (0.0313*C) + (0.0303*D)}}{1 + e^{(-2.0191) + (-0.1407*A) + (-0.1939*B) + (0.0313*C) + (0.0303*D)}}$$

Fig. 4 – A cohort of ECMO-eligible CDHSG registry patients ($N = 1476$) divided into deciles by model-predicted probability of ECMO use (or death without ECMO), showing proportion of each decile that actually received ECMO or not. The low risk (0–20%) group represents 33% of patients. The formula for the model is provided, where covariate $A = 1$ min Apgar, $B = 5$ min Apgar, $C =$ highest PCO_2, and $D =$ lowest PCO_2 during the first 24 h of life. An online calculator is available at mobile.nicutools.org. Reprinted from Jancelewicz et al.[82] Copyright © 2018, with permission from Elsevier.

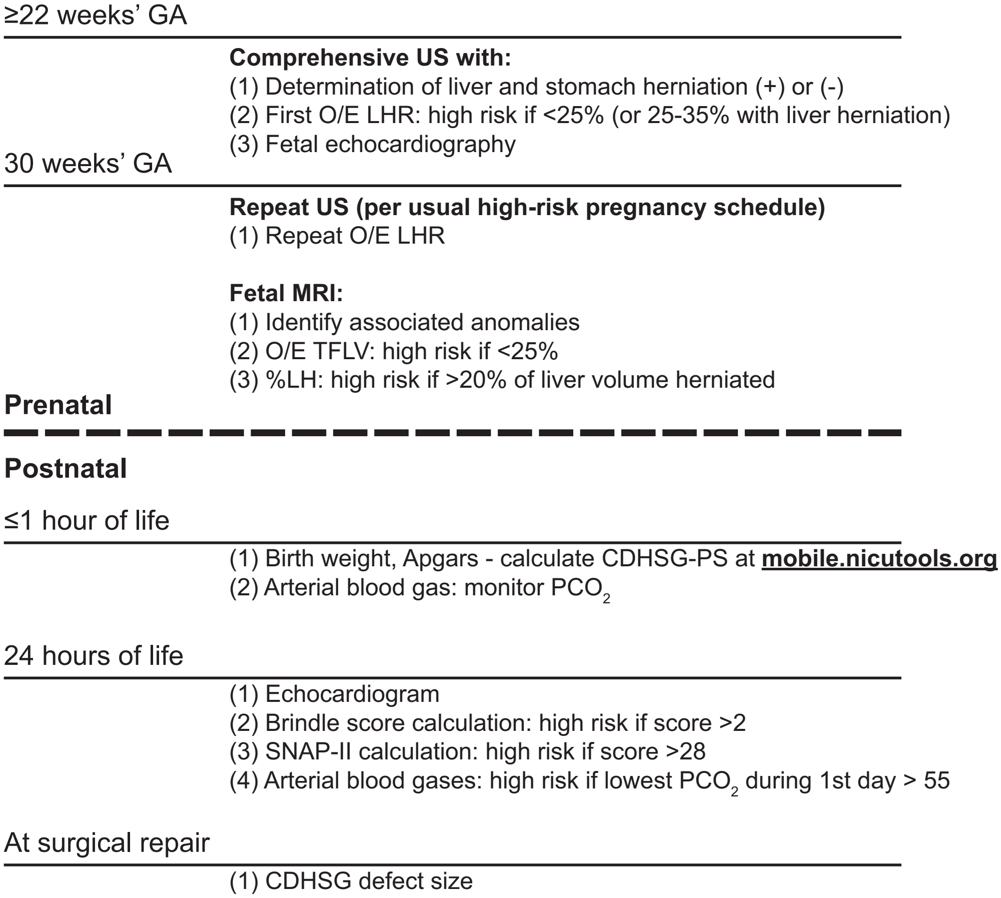
currently be applied to infants prior to operation or to those that do not survive to surgery. Further, it is subjective, with moderate inter-observer variability.[71] This shortcoming has been addressed by development of the detailed definitions for each defect. Despite these downsides, defect size staging remains probably the most accurate CDH risk stratification tool yet developed for the population of CDH infants who survive to surgery.

CDHSG mortality prediction model (Brindle score)

This model, which was developed and internally validated using the CDHSG database in 2014, uses a simple points-based scoring system that is applicable to virtually all infants with CDH and can be quickly calculated at the bedside; variables include birth weight, 5 min Apgar, severe pulmonary hypertension by echocardiogram, and presence of cardiac and/or chromosomal anomaly.[72] The score has been externally validated internationally within population-based studies as well as within individual centers, including those that follow infants treated with fetal therapy.[4,53,73,74] It maintains very good performance metrics across these studies. By design, the model does not include blood gas measurements - a feature that has likely curtailed its predictive potential but has also allowed maximal generalizability across populations. The validated performance characteristics, simplicity and generalizability of this model contribute to its success as a predictive tool.

Echocardiogram

Echocardiographic measures have been developed as individual prediction indices (e.g. the McGoon index) or incorporated into risk prediction models (e.g. the Brindle score). Echocardiography provides information that can aid in risk stratification including: the presence of associated congenital anomalies; estimates of pulmonary hypertension; and a reflection of the cardiovascular status of the patient. Major cardiac anomalies have long been associated with increased mortality in CDH.[75] The infrequency of severe defects, however, makes this finding on its own a poor predictor of mortality. Direct assessments of the pulmonary vasculature that can provide an excellent measure of pulmonary hypertension have been developed as predictive indices. For example, the modified McGoon index measures the combined diameter of the pulmonary arteries at the hilum indexed by the



≥22 weeks' GA

Comprehensive US with:
(1) Determination of liver and stomach herniation (+) or (-)
(2) First O/E LHR: high risk if <25% (or 25-35% with liver herniation)
(3) Fetal echocardiography

30 weeks' GA

Repeat US (per usual high-risk pregnancy schedule)
(1) Repeat O/E LHR

Fetal MRI:
(1) Identify associated anomalies
(2) O/E TFLV: high risk if <25%
(3) %LH: high risk if >20% of liver volume herniated

Prenatal

Postnatal

≤1 hour of life

(1) Birth weight, Apgars - calculate CDHSG-PS at **mobile.nicutools.org**
(2) Arterial blood gas: monitor PCO_2

24 hours of life

(1) Echocardiogram
(2) Brindle score calculation: high risk if score >2
(3) SNAP-II calculation: high risk if score >28
(4) Arterial blood gases: high risk if lowest PCO_2 during 1st day > 55

At surgical repair

(1) CDHSG defect size

Fig. 5 – Guideline for optimal prenatal and postnatal risk stratification in a CDH patient based on the best available evidence. It is likely – but unproven – that multiple or consistent high-risk calculations/observations represent a more accurate risk profile than a single predictor (e.g. prenatal combined with postnatal risk assessment).

descending aorta. In experienced hands, the McGoon index can predict mortality with a high degree of sensitivity and specificity (85% and 100% respectively).[76] This high predictive ability, however, has not been consistently demonstrated across studies.[77] Other measures including pulmonary artery indices are similarly challenged by variable performance. Echocardiographic measures in CDH are most commonly used as dynamic methods of assessing a patient's physiology and are increasingly used in this fashion to predict outcomes and direct decision-making. Systemic and suprasystemic pulmonary pressures are independently associated with adverse outcomes and are typically gauged through findings such as bidirectional or right to left flow through a PDA or PFO, bowing of the intraventricular septum and, eventually, right heart dilation and ultimately hypertrophy. More recently, ventricular dysfunction has been shown to be important independent predictors of outcome (see section 6 of this issue). When combined with other physiologic measures, echocardiography is a strong contributor to risk prediction in CDH.

Other postnatal prediction tools

The application of other postnatal risk stratification tools for CDH has been reported, but, of these tools, only the 12–24-hour Score for Neonatal Acute Physiology, Version II (SNAP-II)[78] has been subsequently validated by other groups.[57,64,67,79] The SNAP-II is somewhat cumbersome to calculate, and the data points are not included in the CDHSG registry, thus it cannot be validated using the largest available cohort. However, the SNAP-II is the only prediction tool that has been assessed as a secondary outcome of a randomized trial, and the C statistic suggests excellent predictive discrimination for survival (0.88) and other adverse outcomes.[79,80] Other accurate but unvalidated single-study survival prediction tools use the best oxygenation index,[81] Pediatric Risk of Mortality III (PRISM III),[55] PaO_2/(fraction of inspired oxygen (FiO2) – PCO_2),[53] and highest preductal PCO_2/FiO_2.[59]

ECMO prediction

Prediction of ECMO use in CDH patients is useful in order to compare ECMO utilization between centers, to ease transition to ECMO for caregivers and families, and to target patients for timely and appropriate use of this invasive treatment.[82,83] It is complex to address because ECMO is an intervention and not an outcome in and of itself. Prenatal imaging and postnatal variables have both been shown to be associated with ECMO use, and have largely been found to mirror the outcome of survival in terms of predictive accuracy.[29,30,54,62,79,84–87] ECMO use, then, can be generally thought of as a surrogate for mortality risk. While highest or lowest PCO_2 during the first day of life predicts survival, it also strongly predicts ECMO use, and in some papers it is a stronger predictor of ECMO use than of survival.[64,82] This implies that failure to ventilate predicts a transition to ECMO more consistently than it predicts overall mortality, and non-pulmonary variables are less predictive of survival in the ECMO population (e.g. birth weight).[56] A recent model derived using the CDHSG registry accurately calculates the probability of ECMO treatment (or death without ECMO treatment) using first-day highest and lowest PCO_2 and 1 and 5 min Apgars (Fig. 4); this tool is available online at mobile.nicutools.org and is useful to compare outcomes between centers.[82,83]

Guideline for use of prediction tools in CDH

There have been several guidelines published that provide brief suggested protocols for optimal risk stratification in CDH patients.[10,88,89] Using these guidelines and the best available evidence examined in this report, we have generated a simple protocol that may be followed, as institutional resources allow, to help clinicians obtain the most valuable prognostic information at each stage of patient care (Fig. 5).

Summary

There are well-established risk prediction tools available for clinicians to generate and confirm an individualized risk profile for every CDH patient beginning early in gestation and extending through the early postnatal period. Accurate stratification is possible with or without access to advanced imaging techniques. Given the variable severity that characterizes this disease, these tools are a vital component of the successful management of CDH patients.

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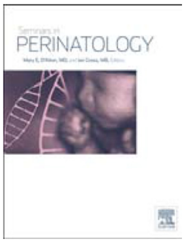

The role of ECMO in the management of congenital diaphragmatic hernia

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ABSTRACT

Congenital diaphragmatic hernia (CDH) is the most common indication for extra-corporeal membrane oxygenation (ECMO) for neonatal respiratory failure. CDH management is evolving with advanced prenatal diagnostic imaging modalities. The risk profiles of infants receiving ECMO for CDH are shifting towards higher risk. Many clinicians are developing and following clinical practice guidelines to standardize and optimize the care of CDH neonates. Despite these efforts, there are significant differences in the practice patterns among ECMO centers as to how and when they choose to initiate ECMO for CDH, when they believe repair is safe, as well as many other nuances that are based on center experience or style. *The purpose of this report is to summarize our current understanding of the new and recent developments regarding management of infants with CDH managed with ECMO.*

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Introduction

CDH is a condition in which the natural barrier between the abdomen and thorax is missing. The resulting pulmonary parenchymal compression affects formation of both lungs at the structural level during fetal development. This compression, in addition to the early embryologic insult, results in infants born with a varying range of hypoplastic and poorly developed lungs. In utero, there is minimal consequence for the developing fetus as placental and fetal circulation allow for normal levels of gas exchange. After birth, immature lungs are not able to meet the demands of adult type circulation. This leads to hypoxia, which further exacerbates pulmonary vascular resistance and causes progressive respiratory failure. If not treated with mechanical ventilation and other interventions, this is a lethal problem for a newborn.

ECMO is a rescue therapy that maintains cardiac and pulmonary function, allowing recovery from a reversible respiratory problem. Neonates with CDH have varying degrees of pulmonary insufficiency, and in the most severe cases infants receive ECMO to support cardiopulmonary function. Up to 30% of neonates with CDH receive ECMO.[1–3] As is the case with all procedures, ECMO risks and benefit must be carefully considered. As reported by the Extracorporeal Life Support Organization (ELSO) Registry, there are about 250–300 infants with CDH per year who go on to develop respiratory failure significant enough to receive ECMO.[4] Roughly half of these infants do not survive.[4] The remaining survivors have a high degree of long-term morbidity such as poor neurodevelop-

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mental outcomes.[5,6] With ongoing clinical and basic science research, there is a significant effort to improve the care of these infants born with CDH. In this review, we sought to highlight those areas where there is alignment in patient care as well challenging aspects with differing opinions on the management of CDH and ECMO.

The history of ECMO in the management of CDH

The early days of neonatal respiratory ECMO centered on the management of persistent pulmonary hypertension and a series of randomized trials followed to test the benefits of this new intervention. The first prospective randomized trial of ECMO in neonatal respiratory failure was conducted by Bartlett et al. who involved an adaptive design using a randomized "play-the winner" method. The trial only had one control patient who died; all survivors were in the ECMO group.[7] There were significant commentaries that followed which criticized the trial, given there was only a single control patient. The UK collaborative conducted a larger trial and showed further benefit towards ECMO in neonates demonstrated by greater survival and lesser degrees of neurodevelopmental disability at 1 year of age.[8] Globally, clinicians soon realized that ECMO regularly resulted in survival in neonatal respiratory failure and many surviving neonates grew up to have a comparable quality of life, which therefore led to wide adoption of ECMO.

The first use of ECMO for CDH was described by German in 1977.[9] This report described repair of CDH on ECMO resulting in the survival of 1 out of 4 infants. There has never been a randomized trial conducted to determine whether the use of ECMO in CDH is beneficial. This is notable because CDH carries the greatest mortality of all common neonatal conditions receiving respiratory ECMO.[10] To date, the UK collaborative trial had the largest number of infants with CDH, where 14/18 in the ECMO group died, and 17/17 in the conventional arm died.[8] Mortality among infants with a primary diagnosis other than CDH was 21% in the same study.[8] Of note, this study was conducted in the late 1980s and survival rates have improved since that time.[11]

In 1997, "A Tale of Two Cities" reported the comparative experience between Boston Children's Hospital and The Hospital for Sick Children, Toronto. According to this report, neither high frequency ventilation nor ECMO significantly improved outcome.[12] Postnatal management strategies have evolved significantly since then, with a strong emphasis on protocolized practice guidelines, postnatal stabilization, gentle ventilation, and multi-modal treatment of pulmonary hypertension. ECMO is presently utilized for the most severe cases when pre-specified limits of medical management have been reached.

Prenatal predictors of need for ECMO in CDH

Approximately 68% of neonates with CDH are diagnosed prenatally.[13] Prenatal prognostic metrics to assess the severity of pulmonary hypoplasia and pulmonary hypertension have been developed. Antenatal parameters such as Lung-Head-Ratio (LHR), observed to expected LHR (O/E LHR), total fetal lung volume (TFLV), observed to expected TFLV (O/E TFLV), percent predicted lung volume (PPLV), and liver to thorax ratio (LiTR) define the degree of pulmonary hypoplasia, while pulmonary arterial hypoplasia/hypertension can be estimated by the McGoon's index or modified McGoon's index. These prenatal factors along with herniation of liver, position of stomach, and lung/liver signal intensity ratio (LLSIR) have been shown to be associated with postnatal survival[14] and are addressed elsewhere in this edition of the *Seminars*.

There are studies specifically examining prenatal predictors of need for ECMO in the CDH population. In a recently completed meta-analysis, Russo et al. found that LHR <1 [RR 1.65 (95%CI 1.27−2.14)], O/E LHR measured by US [pooled standardized mean difference (SMD) −0.73 (95%CI −1.07 to −0.2.14)], and O/E TLV measured by MRI [SMD −1.00 (95%CI −1.52 to −0.48)], as well as liver herniation [RR 3.04 (95%CI 2.23−4.14) significantly predicted the need for ECMO.[15] By itself, intrathoracic position of the liver is also highly associated with ECMO use. Hedrick et al. showed that 80% of fetuses with liver up on prenatal imaging would go on to require ECMO, compared to 25% of fetuses with liver down.[15,16] There is heterogeneity in these measurements and ECMO utilization criteria among different institutions, which may be where some the variability stems from for these measurements. It is certainly reasonable for institutions to self-determine their own cut-off where ECMO is very likely and which infants will benefit from birth at or near the ECMO center.

In addition to lung size and liver position, fetuses with CDH must be assessed for associated structural and genetic anomalies, as the existence of these anomalies could significantly impact the decision to initiate ECMO as well as the overall outcome of the fetus. The ECMO utilization rate for CDH reported to ELSO with concomitant chromosomal abnormalities is rare,[17] yet this demonstrates that it is not an absolute contraindication, and should be a multi-disciplinary institutional decision with a thoughtful informed consent process. The congenital diaphragmatic hernia composite prognostic index (CDH-PI) takes into account numerous antenatal variables and was found to be predictive of not only postnatal mortality, but also the need for ECMO.[18] The CDH-PI consists of a scoring system of 10 parameters that includes karyotype abnormalities, syndromic features, presence of congenital heart disease, left ventricle/right ventricle proportion, modified McGoon's index, presence of hernia sac, liver herniation, LHR, TLV, and PPLV. The authors found that a CDH-PI score of >8 was associated with improved survival (89% vs. 38% for infants with a CDH-PI score of <8). Furthermore, decreasing CDH-PI scores were associated with increasing need for ECMO, where 63% of neonates with CDH with a score of <6 were treated with ECMO, while 75% of those with a score of <5 received ECMO therapy.

Ex utero intrapartum treatment (EXIT) to ECMO

Ex utero intrapartum treatment (EXIT) to ECMO has been proposed as a treatment for severe CDH, i.e. those infants who have an extremely high mortality or ECMO risk based on prenatal imaging. This involves cannulating the infant and instituting ECMO therapy while the infant remains on placental support. The theoretical advantages of such an approach are avoidance of barotrauma, avoidance of hypoxia, minimizing the affects hemodynamic instability and end-organ damage from shock. EXIT to ECMO was initially described by Kunisaki et al., for infants with liver herniation, LHR<1.4 and predicted lung volume <15%. In this study, the EXIT procedure was performed in conjunction with a 20 min ventilation trial. Of the 14 infants in the study, 11 underwent EXIT to ECMO, where 4 died and 7 survived. The results of this study suggested EXIT to ECMO was a feasible option. However, a second analysis by Stoffan et al., didn't support these results as 5/10 infants survived without EXIT to ECMO compared to 2/6 who survived after EXIT to ECMO.[19] The last study that evaluated EXIT to ECMO for CDH sought to determine if there were morbidity differences in survivors.[20] In this study, 8 EXIT to ECMO survivors were compared to 9 non-EXIT survivors and all had mild motor or speech delay without any differences between the groups. These data do not support leveraging EXIT to ECMO as a treatment option for high-risk infants given equivocal outcomes. Based on the data from these studies, EXIT to ECMO is not routinely practiced nor generally recommended.

Pre-ECMO management of CDH patients

Initial management of newborn infants with CDH has significantly evolved over the last few decades and multiple collaboratives have adopted clinical practice guidelines (CPGs).[21,22] Currently, the CDH Study Group is also developing a proposed overarching CPG. Overall, the essential components of CPGs include a well-coordinated initial resuscitation in the delivery room, followed by a protocolized assessment and resuscitation within the neonatal intensive care unit. After delivery, with near term vaginal delivery accepted as safe for infants with CDH, initial resuscitation efforts should include standard newborn care, a secured airway, and alimentary tract decompression with a large orogastric tube. Once transported to the neonatal intensive care unit, a chest radiograph, head ultrasound and an echocardiogram should be obtained. Adequate venous and arterial access must be established. Gentle ventilator management, with pre-specified limits, is employed to avoid barotrauma, volutrauma, and atelectrauma. Preductal saturations and blood gases should be trended and utilized for decisions regarding escalation of support.

Neonates with CDH can progress rapidly from acceptable hemodynamic parameters to cardiopulmonary failure. For neonates with tenuous respiratory status, the use of high frequency oscillatory ventilation (HFOV) is a common next step. It is worth noting that a recent trial was not able to demonstrate a difference in mortality or development of bronchopulmonary dysplasia between conventional ventilation and HFOV.[23] If preductal saturations remain <80%, inhaled nitric oxide (iNO) may be started, though strong data question its efficacy. Although studies have not shown iNO to either avert ECMO or improve survival in the CDH patient population,[2,24] it continues to be commonly used.[25–27] iNO use can be considered for severe pulmonary hypertension with close assessment of patient response, or simply as a bridge to ECMO.[28,29] Based on available evidence, use of iNO in the setting of left ventricular dysfunction may have deleterious effects, such as non-response to vasodilators and occurrence of pulmonary hemorrhage.[30,31] When these collective strategies fail to adequately address severe physiologic derangements of hypoxia, acidosis, hypercarbia, and/or hypotension, ECMO should be considered (Table 1).

Table 1 – Physiologic Derangements Indicative of the Need for ECMO in CDH.

Derangement	Assessment
Hypoxia	Preductal saturations consistently <80–85%
Acidosis	Metabolic (lactate >5 mmol/L or pH<7.20) or respiratory (pH<7.20 due to hypercarbia)
Hypercarbia	Persistent $PaCO_2$ >70 leading to pH <7.20
Hypotension	Poor tissue perfusion, urine output <0.5 cc/kg/hr; unresponsive to IV fluid and inotropic support

Of course, there is institutional variation on ECMO utilization for CDH.[32] Prenatal criteria as discussed above can be useful in predicting severe disease and need for ECMO in order to marshal appropriate resources and help guide initial management. In addition, Jancelewicz et al. have described an ECMO prediction model using postnatal data.[33]

Indications and contra-indications for ECMO in CDH

ECMO is used in infants with CDH experiencing deteriorating clinical status, frequently secondary to pulmonary hypertensive crisis—in theory, a reversible condition. As noted, the indications for ECMO are not uniform. Relative indications for ECMO have been studied by multiple investigators and include elevated oxygenation index (OI), persistently low oxygen saturations despite maximal ventilator assistance, hypercarbia, and elevated alveolar-arterial oxygen gradient (A-aDO2) (Table 2).

The Conventional Mechanical Ventilation Versus High-Frequency Oscillatory Ventilation for Congenital Diaphragmatic Hernia Trial (The VICI-trial) utilized the following predetermined failure criteria as possible indications for ECMO if they were met at two consecutive time points for at least 3 h: 1 - inability to maintain preductal oxygen saturation above 85% (52 mmHg or 7 kPa) or post ductal saturations above 70% (40 mmHg or 5.3 kPa); 2 - increase in $P_aCO_2 > 65$ mmHg or 8.5 kPa despite optimization of ventilatory management; 3 - peak inspiratory ventilator pressure (PIP) > 28 cm H_2O; 4 - mean airway pressure (MAP) > 17 cm H_2O; 5 - inadequate oxygen delivery with metabolic acidosis defined as lactate ≥ 5 mmol/L and pH < 7.20; 6 - hypotension resistant to fluid

Table 2 – Relative Indications for ECMO in the CDH patient.

Author	Criteria for initiating ECMO
Sebald[88]	OI > 40 for 4 h or $PaO_2 < 40$ for 2 h
Boloker[89]	Preductal O_2 saturation <80% refractory to ventilator manipulation (PIP >30 with convention ventilation, MAP of 20 on HFOV)
Somaschini[90]	OI > 40 or $PaO_2 < 40$
Nagaya[91]	Emergent: OI > 40 or $PaO_2 \langle$ 40 or $PaCO_2 \rangle$ 100 for 2 h Preventative: $FiO_2 > 0.9$ or MAP > 12 for 24 h
Vd Staak[92]	A-$aDO_2 > 610$ for 8 h or OI > 40 for 3 of 5 consecutive blood gases
Howell[93]	A-$aDO_2 > 610$ for 8 h or OI > 40 for 2 h

Table 3 – Relative Contraindications to initiating ECMO support in the CDH patient.

Severe and multiple congenital anomalies
Critical cardiac anomalies
Lethal chromosomal abnormalities
Grade III/IV intracranial hemorrhage
Weight < 2 kg
Gestational age <32–34 weeks
Prolonged high pressure mechanical ventilation

therapy and inotropic support resulting in a urine output < 0.5 ml/kg/hour; 7 - oxygenation index ≥ 40.[23] Overall these indications provide a good summary of when ECMO support may be provided for neonates with CDH. However, all are subject to numerous, patient-specific factors and actual patients may meet these criteria for a variable period of time, followed by periods of stability, which can make the decision to initiate ECMO difficult. Other patients may have a more dramatic deterioration making the decision to provide ECMO support the only option.

Contraindications for ECMO support for CDH are also changing as the field is evolving.[34] The generally accepted contraindications for ECMO are: 1 – significant congenital anomalies (e.g. severe cardiac lesions) and lethal chromosomal disorders, 2 - irreversible brain damage, 3 - uncontrolled bleeding; and 4 - intraventricular hemorrhage grade III or greater. Other relative contraindications for infants with CDH include: 1 - <2 kg in weight; 2 - <32–34 weeks gestational age; and 3 - high probability of a poor prognosis (Table 3).

ECMO cannulation in the neonate with CDH

The two methods of delivering ECMO support to the newborn with CDH are venoarterial (VA) and venovenous (VV). VA ECMO is most commonly performed by cannulation of the right carotid artery and jugular vein. VV ECMO is accomplished through placement of a double lumen (DL) cannula into the right internal jugular vein. VV cannulation is not always possible if the jugular vein is not adequate in size to accommodate the smallest VVDL cannula (currently 13 French). VV cannulation is also not an option with extremely poor cardiac function or for extracorporeal cardiopulmonary resuscitation.[35] Mode of cannulation is most often dictated by institutional preference or the perceived degree of illness of the neonate.[32]

A number of studies have compared VV versus VA ECMO for CDH and none have reported a difference in mortality.[4,36,37] These studies were criticized for not being able to adequately control for disease severity or selection bias.[38] To address this, recently a propensity score-based analysis of the ELSO registry was carried out from the years 2000–2016. The propensity score matching identified 3304 infants (VA = 2470, VV = 834) and the odds of death were not significantly different the two groups (OR = 1.01, 95% CI: 0.86–1.18, P = 0.95).[39] Neither was a difference seen in the odds of severe acute neurologic events between the matched VV and VA patients.[39] Subgroup analysis of pre-ECMO CDH repair compared 175 VA with 70 VV cases. In this group, the odds of death for the VV group was more than doubled compared to the VA group (OR = 2.10, 95% CI: 1.19–3.69; P = 0.01). There was no difference in odds of severe neurologic complications within the two groups undergoing pre-ECMO CDH repair (OR = 1.48; 95% CI: 0.59–3.71; P = 0.39). Since this was a subgroup and the number of patients are much smaller, the significance of this finding in the pre-ECMO repair group remains unclear. Other subgroups based on timing of repair were also analyzed and no differences in mortality or acute severe neurologic events were noted.[39]

Clearly, these studies all have limitations related to the source of data which can limit matching methodologies. Therefore, conclusions drawn are not equal to a well-designed randomized controlled trial. What can be recommended based on the evidence are the following: 1 - many prefer VA ECMO and have a bias for VA ECMO when the neonate is deemed to be more critical; 2 - VA ECMO should be used in cases of sudden arrest or extremely poor cardiac function; and 3 - assuming vessel size is not the limiting factor, VV and VA have similar outcomes and the modality selected should be based on individual and intuitional experience or preference. Table 4 summarizes the advantages of these two modalities of ECMO support.

In the setting of VV ECMO, some centers routinely place an additional cephalad jugular venous catheter.[40] The reasoning behind this is to reduce cerebral venous pressure and decrease the risk of intracerebral hemorrhage. Based on ELSO data, this practice has not been shown to decrease the risk of neurologic complications or survival for VV cannulation in the neonate with CDH.[41] Thus, the decision to place a cephalad drainage catheter should be based on individual patient factors, equipment availability and center experience.

Challenges to ECMO cannulation

Due to the complex of anatomic challenges in infants with CDH, cannulation can be more difficult compared to ECMO cannulation for neonatal respiratory failure for other reasons (Fig. 1). For example, infants with CDH have been described to have hypoplastic neck vasculature which may limit the size of the cannula, rendering VV challenging, high-risk, or impossible.[42] Bicaval double lumen cannulas are associated with

Table 4 – Advantages of venvenous (VV) and venoarterial (VA) ECMO in CDH.

Advantages of VV ECMO support	Advantages of VA ECMO support
Provides oxygenated blood to pulmonary vasculature and myocardium	Provides cardiac / hemodynamic support
Does not disrupt the carotid artery	Initiation usually not limited by small vessel diameter
Preserves pulsatile flow	Decreases preload
Stabilizes preload and afterload	Management more straightforward – less susceptible to flow and position challenges
Decreased risk of cardiac stun	No need to urgently convert mode of support
Minimal neurologic embolism risk	

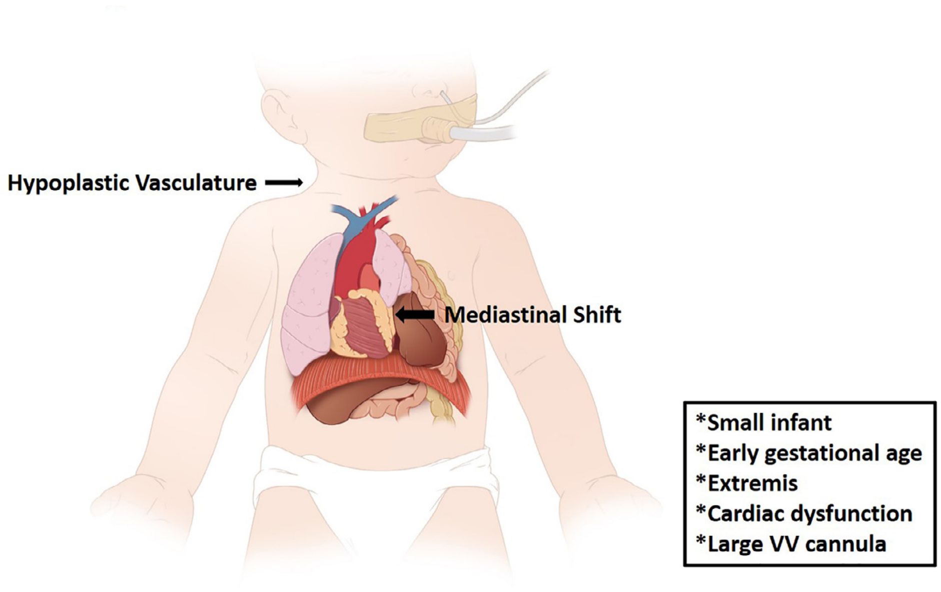

Fig. 1 – Potential Challenges to ECMO Cannulation in the Neonate with CDH.

significant challenges for accurate placement and complications during cannulation have been reported.[43,44] Right-sided CDH presents additional challenges given their unique thoracic anatomy and mediastinal shift due to liver position in the right thorax. As a result, the venous cannula may preferentially enter the ostium of an ectatic azygous vein, which can be difficult to recognize and correct.[45,46] Beyond these anatomic challenges, there is no evidence that VV or VA cannulation is superior in infants with right-sided CDH.[4,39] A recent APSA (American Pediatric Surgical Association) survey study revealed a preference for VA ECMO in right-sided CDH patients, which may be due to the perception of increased severity of right-sided CDH.[32]

Pump type

ECMO circuits are powered by one of two types of pumps: roller or centrifugal. The roller pump works by compressing the tubing, thereby propelling blood forward via positive displacement; simultaneously, blood from the venous reservoir is pulled into the tubing due to negative pressure. The advantage of the roller pump is constant antegrade flow and reduced hemolysis at the low flow rates required in neonates. The disadvantage of the roller pump is that it will continue to rotate independent of the pressure in the circuit or the volume of blood in the venous reservoir. Thus, it requires servo-regulation mechanisms to ensure that circuit volumes and pressures do not exceed safe levels. Furthermore, wear on the tubing and even raceway tubing rupture have been reported.[47]

The centrifugal pump is being increasingly used in ECMO.[48] This pump employs a spinning magnetic disc that creates a constrained vortex. Negative pressure is therefore generated, pulling blood into the pump and then out of the top of the vortex. The centrifugal pump eliminates repeated compression of the tubing, reduces the risk of excessive negative pressure, and decreases priming time and volume.[49] The two main disadvantages of this pump are the difficulty in maintaining set flow and the increased incidence of red blood cell hemolysis from turbulent flow in the pump head vortex. Hemolysis can lead to hyperbilirubinemia, acute renal failure, and other end-organ damage.[49]

There is only one study examining the relationship between ECMO pump type and outcomes in the CDH population. Using the ELSO registry, Delaplain et al. compared roller pumps to centrifugal pumps with a propensity matched cohort. In their study, PS matching identified 1808 infants (centrifugal = 904, roller = 904). There was no difference in mortality or severe neurologic injury between the two groups.[49] There was at least a six-fold increase in odds of hemolysis for centrifugal pumps in all groups.[49] Despite these findings, many centers are using centrifugal pumps for neonatal ECMO.

Surgical repair

Timing on ECMO

The optimal time to perform surgical repair of the diaphragm after birth has long been an area of interest and considerable debate. Emergent repair was thought to be necessary for decades due to the belief that decompressing the lung by removing the herniated abdominal contents from the chest was crucial to improve respiratory distress. Several reports of successful preoperative stabilization prior to repair were reported in the 1980s, showing equivalent to improved survival.[50,51] Lung compliance was even shown to worsen after surgical repair rather than improving.[52] By the 1990s, delayed repair after a period of stabilization had become a widely accepted approach.[53–57] As described previously, a strategy of low pressure *gentle* mechanical ventilation with permissive hypercapnea is employed until sufficient stability for an operation has been demonstrated.[58] Repair is commonly delayed for 24–72 or more hours.

Up to 30% of patients with CDH remain unstable despite optimal medical management and require ECMO cannulation.[59,60] There is no consensus on the ideal timing of surgical repair in the setting of ECMO. Arguments have been made for early repair on ECMO, late repair on ECMO, or waiting until the infant is able to be decannulated from ECMO prior to repair. As repair on ECMO entails performing the operation while the infant is fully anticoagulated, the risk of hemorrhage during or after repair is central to this discussion.[61] Proponents of waiting to repair until after ECMO have argued that not only is the risk of significant bleeding less, but that repair itself has not been shown to improve pulmonary hypertension or respiratory failure. The ELSO Congenital Diaphragmatic Hernia Interest Group and the Congenital Diaphragmatic Hernia Study Group have each reported a survival benefit to delaying CDH repair until after decannulation from ECMO.[62,63] Similarly, Robertson et al. reported a survival benefit for repair after ECMO when they evaluated their institutional experience.[64] In this comparison of their evolving institutional strategy, survival in patients able to be decannulated prior to repair was 94.4% compared with just 43.3% in the early repair group, despite the early repair strategy being the more recent one adopted. Moreover, they found that the early repair strategy was associated with longer ECMO duration. Partridge et al. also found that in their institutional experience, repair after ECMO was associated with increased survival (100%), lower rates of surgical bleeding and decreased duration of ECMO compared with repair on ECMO (survival 43.9%).[65] Of note however, 16 patients of 77 did not survive to repair. If repair confers any benefit, it is plausible that some of these patients may have survived if repaired earlier.

Early repair appeals to the intuition. By removing herniated contents from the chest and recreating normal anatomy, the compressed lungs may expand and contribute more to gas exchange and perhaps pulmonary hypertension may also thereby be improved. If bleeding could be avoided, early repair may thus reduce duration of ECMO. Those who favor this approach have also argued that bleeding risk may be less in the period immediately after ECMO initiation compared with weeks later when tissue edema may be worsened. Dassinger et al. reported that of 34 patients repaired on ECMO in their institution a median of 1 day after cannulation, 71% survived with an 8.8% rate of significant perioperative bleeding (and none of these leading to death).[66] Similarly, when Fallon et al. compared their evolving strategies of early repair (<72 h), late repair (>72 h), and post-decannulation repair, they found a survival benefit to early repair (73% vs. 50% late, and 64% post) despite those repaired early having worse prenatal factors.[59] Those repaired early were also found to have a shorter duration of ECMO by 6 days. Bleeding risk was similar. A more recent study out of the CDH Study Group also found that survival in those repaired early (<72 h), survival was 87%; however, in this study the duration of ECMO was longer.[67]

A strategy of late repair on ECMO can be viewed as a compromise between these two approaches. Repair is performed on ECMO, typically after the patient has been proven ready to decannulate. If all goes well, the patient may be decannulated the following day. If physiologic parameters worsen as a result of perioperative stress, ECMO is already in place as a safety-net, sparing the need for a second more risky cannulation in the contralateral neck (and the eventual ligation of bilateral carotid arteries and jugular veins after decannulation). On the other hand, if hemorrhage occurs during or after surgery, the patient in theory may be decannulated, obviating the need for anticoagulation. One problem with this approach is that not all patients will reach the point of readiness to decannulate soon after repair. This strategy also begs the question: how often is repair so hemodynamically destabilizing that re-cannulation to ECMO is required? A recent study indicates this may be less of concern than previously thought: of 668 patients in the CDH Study Group database who were repaired after decannulation, only 6 (0.9%) required recannulation.[68]

Thus, a consensus on the best time to repair the diaphragm in relation to ECMO has not been reached and practices vary.[69] More work to help identify patients who would most benefit from early repair is needed. Coming full circle to original management strategies, Kays et al. have even argued recently that urgent early repair within the first few hours of life *before* anticipated ECMO may offer the best chance of survival in the most severe cohort of left-sided liver-up CDH patients.[70]

Finally, a recent investigation from the CDH study group evaluated 1581 patients with CDH who received ECLS, performing two comparisons: 1. repair on ECLS compared to repair after ECLS and 2. repair early on ECLS compared to repair later on ECLS. These groups were matched based on propensity score and, importantly, the study accounted for non-repaired patients. CDH repair on ECLS resulted in a 46% reduction in mortality compared to repair after ECLS while early repair on ECLS was associated with a 49% reduction in mortality compared to late repair on ECLS.[71] While resource intensive and optimal with appropriate experience and expertise, it appears that early repair, on ECLS, affords the best survival for all patients on ECLS.

On-ECMO repair strategies

As discussed, the primary concern with surgical repair on ECMO is bleeding due to anticoagulation. Hemorrhagic complications can be fatal. Different strategies have been used to reduce this risk. First, the level of anticoagulation should be decreased temporarily at the time of surgery.[72] Use of thromboelastography (TEG) to guide anticoagulation management perioperatively in CDH patients, already described in post-cardiopulmonary bypass patients, is an emerging area of study and could be useful in the management of anticoagulation during repair.[73] Maintaining adequate platelet count, typically >100,000 perioperatively, has been described as another component of a successful perioperative strategy to limit bleeding.[72]

Addition of anti-fibrinolytic therapy has been shown to reduce surgical bleeding events on ECMO.[74] A 10-year single-institution review found that use of Aminocaproic acid (Amicar) significantly decreased the rate of surgical site bleeding.[75] In this protocol, Amicar was administered as a 100 mg/kg IV bolus around cannulation and continued at a 30 mg/kg/h infusion for 72 h or beyond if continued bleeding was encountered. A specific study looking at Amicar during on-ECMO CDH repair is lacking. Use of perioperative tranexamic acid (TXA) has been specifically studied in the CDH population.[76] Using a protocol of 4 mg/kg bolus 30 min to repair and infusing 1 mg/kg/h for 24 h postoperatively, authors found significantly less surgical site bleeding and need for blood transfusions.[76] Anti-fibrinolytic medications can also have thrombogenic effects on the circuit, which must be balanced with the needs of the patient. The condition of the circuit should additionally be considered before these agents are used.

The surgical techniques used during on ECMO repair can also contribute significantly to decrease bleeding risk. Although there are no studies published to describe differences in surgical technique, minimizing dissection of the posterior rim of diaphragm can decrease bleeding, as can liberal use of electrocautery and even argon beam coagulation. The use of a patch should also be considered, given primary repair of certain defects may require significant dissection of the posterior rim of the diaphragm. Temporary abdominal closure is another useful method to avoid abdominal compartment syndrome secondary to bleeding in these tenuous patients; this technique can also allow for packing of any bleeding sites.[77] Topical hemostatic agents such as Surgicel®, thrombin, and Gelfoam® may also be considered. The use of pledgeted sutures may help attain hemostasis at suture sites.

ECMO and CDH survival

Survival rate of infants with CDH treated with ECMO is 50%, a figure that has minimally changed over the last few decades. However, upon closer examination, the modern CDH population demonstrates a higher risk profile than in the past.[11] We postulate that improvements in medical management have shifted the application of ECMO to the highest severity infants. Whether ECMO improves survival in infants with CDH remains a critical question. As discussed earlier, there has never been a randomized controlled trial specific to CDH and ECMO. Therefore, this question cannot be answered directly. However, evidence from the literature would suggest that ECMO does indeed help those infants who may not otherwise survive.[78]

Postnatal determination of mortality risk is a useful means to understand the trajectory of patients. There have been several risk models developed to determine risk of mortality in infants with CDH. These prediction tools are addressed in detail in this edition of *Seminars*. However, none of the established risk models can adequately predict risk of mortality once ECMO is required. Thus, on-ECMO mortality risk models have been developed. One such score is the Pittsburgh Index for Pre-ECMO Risk (PIPER+) specific for venoarterial (VA) ECMO, which can be used to predict on-ECMO mortality risk for infants with CDH needing VA ECMO.[79] We have also developed an ELSO-based CDH-specific risk model to predict mortality before and during ECMO.[17] The on-ECMO CDH score can be used to determine risk of mortality while on ECMO and is the most accurate risk model for CDH neonates needing ECMO.

The reasons why infants do not survive after initiation of ECMO is complex and is very center specific.[80] The primary determinant of ECMO-specific mortality and development of complications is the duration of ECMO.[79,81,82] Duration of ECMO support is individualized for each CDH patient and depends on their unique risk profile as well as the specifics of the treatments they have received. There are no established guidelines as to how long ECMO support should be provided, but the survival rates start to decline significantly beyond 4 weeks to 10−20%; beyond 5 weeks it is only <5−18%.[17,83]

Future therapies

One prenatal therapy that shows promise in decreasing the need for postnatal ECMO in the CDH population is fetoscopic endoluminal tracheal occlusion (FETO). FETO has been shown to feasible and safe in the most severe CDH patients with LHR <1 and liver herniation.[84] The use of FETO is being further investigated and its use may increase in the future.[85] Other potential treatment strategies may include pumpless arteriovenous ECMO and the artificial placenta.[86] These therapies may individually and/or collectively, represent breakthroughs in the management of this complex patient population. Lastly, other future therapies may involve in-utero delivery of growth factors, specific cell or sub-cellular material and gene therapy. Circuits are becoming more simplified and monitoring is becoming more sophisticated. In the future ECMO cannulation for infants with CDH may be done at non-ECMO centers then transported to an ECMO center using compact ECMO pump systems such as PediMag or CentriMag.[87]

Conclusion

As new therapies evolve for CDH, the need for ECMO may decrease and could eventually become obsolete. This is similar to the decline of ECMO utilization for neonatal respiratory distress syndrome. Nevertheless, it is currently a lifesaving intervention that allows time for pulmonary vasculature stabilization, novel therapy application, surgical decompression of the pulmonary parenchyma, and cardiopulmonary recovery. Thus, it is unlikely that ECMO will be completely replaced in the treatment of CDH. What is more likely is that the risk curves will augment further, and ECMO utilization will be targeted to the sickest CDH infants with highly hypoplastic lungs, refractory pulmonary hypertension, ineffective fetal tracheal balloon occlusion, or infants with concomitant complex cardiac malformations. We may also see a shift towards increasing use of ECMO for more premature infants with improved anticoagulation management and the development of smaller cannulas, as well as pumpless AV ECMO. Ongoing research efforts to standardize care and direct the effects of our therapies must remain at the forefront. These efforts will improve and affect future applications of ECMO therapy for CDH.

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Congenital diaphragmatic hernia-associated pulmonary hypertension

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ABSTRACT

Congenital diaphragmatic hernia (CDH) is a neonatal pathology in which intrathoracic herniation of abdominal viscera via diaphragmatic defect results in aberrant pulmonary and cardiovascular development. Despite decades of study and many advances in the diagnosis and treatment of CDH, morbidity and mortality remain high, largely due to pulmonary hypertension (PH), along with pulmonary hypoplasia and cardiac dysfunction. In patients with CDH, hypoplastic pulmonary vasculature and alterations in multiple molecular pathways lead to pathophysiologic pulmonary vasculopathy and, for severe CDH, sustained, elevated pulmonary arterial pressures. This review addresses the multiple anatomic and physiologic changes that underlie CDH-associated PH (CDH-PH), along with the multimodal treatment strategies that exist currently and future therapies currently under investigation.

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Introduction

Despite advances in early diagnosis and treatment, congenital diaphragmatic hernia (CDH) continues to pose a challenge to pediatric intensivists, neonatologists, and surgeons. While there have been improvements in overall morbidity and mortality over the last few decades, advances have been incremental, secondary to a complex, early embryologic pathophysiology that has proven largely refractory to numerous therapies. Mortality of live-born infants with CDH currently approaches 25–30%, with many non-survivors succumbing to CDH-associated pulmonary hypertension (CDH-PH). Understanding the basic mechanisms and altered anatomy that lead to CDH-PH is paramount in targeting future research to improve outcomes.

What is pulmonary hypertension (PH)?

Pulmonary hypertension (PH) is defined as sustained, supranormal pulmonary arterial pressure. This results in dysfunctional pulmonary circulation and suboptimal gas exchange with subsequent decreased oxygenation, ventilation, and/or cardiac function.[1,2] PH is generally classified as primary (idiopathic) or secondary (resulting from a variety of distinct disease entities).[2] The World Health Organization (WHO) classifies PH into 5 main categories/groups: 1. Pulmonary arterial hypertension, 2. Pulmonary venous hypertension due to left heart disease, 3. Pulmonary hypertension due to lung disease and/or hypoxia, 4. Chronic thromboembolic pulmonary hypertension, and 5. Pulmonary hypertension with unclear multifactorial mechanisms (Table 1).[2,3] Traditionally, PH has been diagnosed via right heart catheterization at rest

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Table 1 – World Health Organization classification of pulmonary hypertension.[2,3]

- Group 1 Pulmonary arterial hypertension (PAH)
  - 1.1 Idiopathic (IPAH)
  - 1.2 Heritable (HPAH) – *BMPR2, ALK1, ENG, SMAD9, CAV1, KCNK3*
  - 1.3 Drug and toxin induced
  - 1.4 Associated with (APAH) - connective tissue diseases, Human immunodeficiency virus (HIV) infection, portal hypertension, congenital heart disease (CHD), schistosomiasis
- Group 1′ Pulmonary veno-occlusive disease (PVOD) or pulmonary capillary haemangiomatosis (PCH)
- Group 1′′ Persistent pulmonary hypertension of the newborn (PPHN)
- Group 2 Pulmonary hypertension due to left heart disease
  - 2.1 Left ventricular systolic dysfunction
  - 2.2 Left ventricular diastolic dysfunction
  - 2.3 Valvular cardiac disease
  - 2.4 Congenital/acquired left heart inflow/outflow obstruction and congenital cardiomyopathies
- Group 3 Pulmonary hypertension due to lung disease and/or hypoxemia
  - 3.1 Chronic obstructive pulmonary disease (COPD)
  - 3.2 Interstitial lung disease (ILD)
  - 3.3 Other pulmonary disease with mixed restrictive and obstructive pattern
  - 3.4 Sleep-disordered breathing
  - 3.5 Alveolar hypoventilation disorders
  - 3.6 Chronic exposure to high altitude
  - *3.7 Developmental abnormalities / lung disease**
- Group 4 Chronic thromboembolic pulmonary hypertension (CTEPH)
- Group 5 Pulmonary hypertension (PH) with unclear multifactorial mechanisms
  - 5.1 Haematological disorders - myeloproliferative disorders, splenectomy
  - 5.2 Systemic disorders - sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis
  - 5.3 Metabolic disorders - glycogen storage disease, Gaucher disease, thyroid disorders
  - 5.4 Others - tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

* Group/category for CDH.

and is defined by a pulmonary artery pressure (Ppa) $\geq$25 mmHg (mean).[2]

There is no standard, accepted definition of congenital diaphragmatic hernia-associated PH (CDH-PH). Generally, CDH-PH is caused by aberrant prenatal pulmonary vasculature development, resulting in pathophysiologic postnatal cardiopulmonary circulation. This results in elevated right-heart pressures, followed by circulatory shunting, poor ventilation, and decreased oxygenation. In CDH-PH, the pulmonary vasculature demonstrates hypertrophic smooth muscle cells and vessel thickening (particularly the media), decreased angiogenesis, and altered molecular signal responsiveness.[4,5] Traditionally, CDH-PH is defined using echocardiography, which identifies elevated right-heart pressures relative to the systemic blood pressure. CDH-PH falls under the category of secondary PH, and is best classified as PH related to lung disease or hypoxia (WHO group 3).

The clinical changes noted in CDH and CDH-PH patients can be explained by a *dual-hit hypothesis*.[7] The first hit being aberrant pulmonary parenchymal and vascular development during embryogenesis, and the second being ongoing inhibition of growth/maturation secondary to external compression by abdominal viscera herniated into the thorax. Though no single, fundamental, underlying mechanism has been identified, teratogenic, genetic, nutritional, and sporadic etiologies have all been investigated. It is likely that the true pathophysiology is multifactorial in nature. The following sections will discuss the established and emerging data about the natural course and therapeutic interventions regarding CDH-PH.

Molecular origins of CDH-PH

Multiple molecular pathways have been implicated in the pathogenesis of CDH-PH. Establishing the exact relationship of these signaling cascades (retinoic acid, nitric oxide, endothelin, vascular endothelial growth factor, and others) is difficult given the multifactorial nature of fetal lung and vascular development. Despite multiple scientific models investigating these pathways, most of the findings presented below remain as *associations* undergoing active investigation.

Retinoid acid signaling

Retinoic acid signaling is a key component of both lung and diaphragmatic embryogenesis. Defective retinoid signaling has been implicated in abnormal diaphragmatic development and aberrant pulmonary development.[6] Nitrofen (2,4-dichloro-4′-nitrodiphenyl ether) is a retinal dehydrogenase inhibitor that, when pregnant animals are exposed to at a specific time in gestation, results in CDH and pulmonary vascular anomalies similar to those in humans with CDH.[7] These pathophysiologic anomalies include pulmonary arterial thickening, decreased pulmonary vascular density, and irregular responses to pulmonary vasodilators.[8,9]

In humans, the data on anomalous retinoic acid signaling and CDH-PH are limited to several small series. Major and coworkers investigated serum levels of retinol and retinol binding protein (RBP) in 11 newborn infants with CDH and 11 controls, as well as the mothers of 7 with CDH and 7 controls.[10] Serum levels of both retinol and RBP were 50% lower among infants with CDH and, notably, retinol levels in CDH mothers were significantly higher when compared to controls. These data implicate the placenta or placental transport of retinol during embryogenesis as a potential focal point for aberrant pulmonary and diaphragmatic malformation in CDH. A second, case-control study evaluated maternal vitamin A intake, serum retinol, and serum RBP, comparing CDH and control pregnancies.[11] Although, vitamin A intake $<800\,\mu g$ among normal weight mothers was associated with a significantly increased risk of CDH, no significant difference among all (including over/under weight) mothers was identified. Despite these associations in the literature, further investigation is necessary to fully understand the role of retinoic acid pathways in the development of CDH-PH in humans.

Nitric oxide

Nitric oxide (NO) is a potent vasodilator in the cardiopulmonary vasculature. It plays an important role in embryologic angiogenesis, pulmonary development, and physiologic vascular response. Animal and human studies in CDH show that there is decreased responsiveness to the NO signaling pathway in the pulmonary vasculature. However, despite the recognition that NO signaling is likely altered in CDH-PH, data remains mixed.[9,12–16] Certain studies have demonstrated elevated levels of endothelial NO synthase (eNOS), the enzyme responsible for NO expression in the vascular endothelium, while other studies have found decreased levels of eNOS. Most notably, large retrospective studies have failed to show benefit (either via survival or rescue from extracorporeal life support) with inhaled NO (iNO),[17,18] rendering this once promising therapy likely ineffective. Additional research is needed to completely elucidate the function of NO signaling in CDH-PH.

Endothelin signaling

The endothelins are a family of vasoconstrictive proteins that play a critical role in cardiovascular and pulmonary disease. Endothelin-1 (ET-1), one of three isoforms of this protein, acts primarily on the receptors ET_A and ET_B, influencing pulmonary circulation by balancing vasoconstriction and dilation.[19] ET_A mediates vasoconstriction by stimulating cytosolic calcium release on pulmonary arterial smooth muscle cells, while ET_B causes vasodilation by stimulating the release of prostacyclin and nitric oxide. Normally, the pulmonary vasculature has the highest levels of ET-1 in the body, and patients with PH usually have elevated levels of ET-1 in their lungs and pulmonary vasculature.[19–21] Additionally, studies have found that, in PH, there is an imbalance of endothelin receptors, where a predominance of ET_A compared to ET_B exists. These abnormalities, increased ET-1 and ET_A, shift the system toward vascular constriction. In infants with CDH, higher levels of ET-1 were associated with higher pulmonary vascular pressures and more severe PH.[22,23] Post-mortem studies of infants with severe persistent pulmonary hypertension of the newborn (PPHN) and CDH show significantly elevated levels of ET-1. These associations make endothelin expression and signaling an important therapeutic target in CDH-PH.

Vascular growth factors

Vascular endothelial growth factor (VEGF) has a known role in angiogenesis and endothelial cell proliferation, so it is a key driver of embryologic pulmonary development. Unfortunately, despite multiple investigations, the role of VEGF in CDH-PH remains unclear. VEGF expression has been shown to be either increased or decreased in the nitrofen CDH model.[24,25] VEGF receptor (VEGFr) expression has been shown to be decreased in the same model.[26] Despite these data, human post-mortem studies have shown increases in VEGF, possibly in response to decreased VEGFr expression and hypoplastic vasculature.[27]

Macrophage inhibition factor

Macrophage migration inhibitory factor (MIF) is a trimer protein expressed in vascular endothelial cells, vascular smooth muscle cells, fibroblasts, and macrophages. MIF is a pro-inflammatory cytokine that inhibits random migration of macrophages, has anti-apoptotic activity, and stimulates cell proliferation. MIF levels are 10-fold higher in infants than in children and adults.[28] Hypoxia enhances MIF expression, resulting in fibroblast, endothelial cell, and vascular smooth muscle proliferation leading to vascular wall thickening and PH. In the nitrofen-model of CDH-PH, prenatal inhibition of MIF led to improvement in right ventricular systolic pressure (RVSP) as well as decreased pulmonary vascular wall thickness and cellularity.[28] Additionally, inhibition of MIF was associated with increased VEGF expression in the same model. This was associated with improvement in lung development and pulmonary angiogenesis.

MicroRNAs

Recent studies suggest that MicroRNAs (miRNAs) play a role in the pulmonary dysfunction associated with CDH-PH. miRNAs are non-coding RNAs that promote mRNA degradation and disrupt translation, ultimately suppressing gene translation. Seven miRNAs were differentially expressed in a series of CDH newborns who developed chronic lung disease, suggesting that miRNA expression may correlate with pulmonary dysfunction.[29] More specifically, the let-7 family of miRNA and miR-210 have been implicated in cardiopulmonary diseases associated with scleroderma, HIV, pulmonary hypertension, and ischemic heart disease. While these seven miRNAs were predicted to affect over 400 different genes, a handful of molecular pathways, specifically those involved with extracellular matrix (ECM) organization, cell cycle regulation, and tissue homeostasis, were heavily affected. There has been extensive study on the expression and role of miR-200b in CDH. miR-200b is part of the miR-200 family of epithelial markers that has been implicated in the epithelial-mesenchymal transition in pulmonary fibrosis and some cancers.[30] There is decreased expression of miR-200b among nitrofen-model CDH pups, and this is associated with dysfunctional alveolar branching. Increasing miR-200b in these pups restored the normal branching morphogenesis *ex vivo*.[30] Most promising is that, in the nitrofen-rodent model, prenatal treatment with miR-200b decreased the incidence of CDH overall.[30] Additional study is needed, but micro-RNAs represent an exciting therapeutic opportunity to both decrease CDH incidence prenatally and mitigate its severity in CDH-infants. Moreover, there is ongoing study about using differential miRNA expression as a prognostic biomarker in CDH (see below).[29]

Vascular remodeling

Multiple molecular pathways have been implicated in the pulmonary vascular remodeling that is found in CDH-PH. Pulmonary vascular remodeling is thought to result from a combination of impaired endothelial cell function combined with imbalanced signaling to pulmonary arterial smooth muscle cells (PASMCs). Aberrant signaling can happen anywhere in the molecular roadmap of fetal lung development. For example, multiple studies have shown decreased expression of VEGF receptors in CDH-infants, resulting in elevated plasma levels of VEGF via the negative feedback loop.[31] Elevated VEGF contributes to altered cell signaling and vascular development, and can be predictive of PH and mortality. Moreover, bone morphogenic protein (BMP) pathway signaling is blunted in CDH pups using the nitrofen-rodent model. This affects downstream BMP targets such as Apelin (APLN), which normally attenuates the PASMCs response to growth factors. Additionally, APLN and its receptor are both downregulated in the nitrofen-rodent model, resulting in disrupted endothelial homeostasis. The VEGF, APLN, and BMP pathways are just a few of many different molecular pathways that have been implicated in impaired respiratory vascular homeostasis. The resultant endothelial cell (EC) dysfunction involves cellular immaturity, decreased expression of transcription factors necessary for EC function and angiogenesis, and impaired signaling to PASMCs.[31] This, in turn, leads to PASMC proliferation and resistance to apoptosis. Clinically, these phenomena present as the hypertrophic pulmonary arterioles often found in CDH-PH.[31]

Anatomy and histology of CDH-PH

Anatomic and histologic irregularities have been identified in the pulmonary vasculature in CDH-PH. Grossly hypoplastic ipsilateral and mildly hypoplastic contralateral lungs have been found on post-mortem analyses.[32] Other abnormalities identified included small bronchi, decrease bronchiole generations (50% fewer), and a decrease in pulmonary arteriole number, size, and branching.[32,33] The muscularization of small arterioles and hypertrophy of the pulmonary artery are well described in CDH-PH.[32,33] Rodent and human models have both demonstrated medial and adventitial wall thickening and displacement of vascular smooth muscle cells.[5,34,35]

Vascular development

CDH is associated with aberrant pulmonary vascular development, postulated to start as early as 4–16 weeks gestation. Normal lung development starts with the embryonic lungs branching into lung buds and then developing the terminal bronchioli. Alveoli are formed from 24 weeks gestation until 3 years of age. The pulmonary vasculature develops along with the airways, and normally occurs through a process of distal angiogenesis. Distal angiogenesis involves the branching of new capillary endothelium at the tips of pre-existing vessels. This is followed by stabilization of the endothelium by pericytes and other perivascular cells.[34] These processes are mediated by well described molecular pathways involving endothelin. The increased expression of ET-1 and its receptors, specifically ET-A, in CDH are likely to contribute to the pathologic proliferation of vascular smooth cells in pulmonary vessels. Moreover, human and rodent CDH-PH tissue shows increased expression of the ECE-1 enzyme, which is key in the conversion of ET-1 to its active form. This suggests that not only is there an increased level of circulating ET-1, but also potentially increased bioavailability. There is ongoing study into the roles of the retinoic acid, micro-RNAs, and eNOS pathways in vascular development in CDH.[35]

Vasoactive pathways and unresponsiveness

Despite the identification of multiple pathways involved in the pathogenesis of CDH-PH, treatment strategies targeting these pathways remain largely unsuccessful. These same therapy options, including iNO and sildenafil, among others, have worked well for pulmonary hypertension of other etiologies. This suggests that the vasoactive signaling pathways in CDH-PH are uniquely unresponsive to targeted therapies. Altered or unique receptor expression, alterations in gut absorption, and increased drug metabolism, along with unconventional pathway alterations, all contribute to the difficulty of finding safe and effective CDH-PH therapy.[36]

The levels of pulmonary pathophysiology, including the levels of the organ, tissue, cellular, and subcellular, in CDH-PH are shown in Fig. 1.

Defining, diagnosing, and following CDH-PH

In children, PH is defined as a resting mean pulmonary artery pressure (mPAP) >25 mmHg beyond the first few months of life.[3] Given that CDH-PH manifests acutely (in utero or in the immediate postnatal period), and that cardiac catheterization is an invasive procedure, this “gold standard” definition of PH is suboptimal for CDH patients.

Echocardiography is non-invasive, avoids radiation exposure, provides an excellent evaluation of cardiac structure and function, and can be used as needed to monitor disease progression or response to therapy, making it the preferred screening modality for CDH-PH.[37,38] The pulmonary artery pressure (PAP) can be estimated by evaluating the velocity of the tricuspid regurgitation (TR) jet, which determines the right ventricle (RV) systolic pressure (SP) and reflects the PAP (assuming physiologic RV outflow) through the modified Bernoulli equation.[3] Systemic (systolic) blood pressure (SBP) is captured simultaneously to determine the ratio of the PAP to the SBP. Despite the utility of echocardiographic measures, the TR jet is inadequate to assess the velocity in 10–25% of patients with PH.[3,39]

Secondary signs of PH should also be assessed. Leftward displacement, bowing, or flattening of the interventricular septum occurs when the RVSP eclipses $\frac{1}{2}$ of the SBP.[3,40] LV ejection fraction (LVEF) and LV wall thickness may be affected by septal displacement.[41] RV wall thickness (hypertrophy), RV volume (dilation), and RV ejection fraction are often abnormal in PH, though they can be difficult to assess given their non-geometric shape and the anatomic position of the RV.[3] Finally, intracardiac (atrial-septal defects (ASD) or ventricular-septal defects (VSD)) and/or extracardiac (patent ductus arteriosus (PDA)) shunts should be assessed, including the size of the shunt, along with the direction and severity (velocity) of flow.[3] Identification of right-to-left or bidirectional flow indicates increased right-sided cardiac pressures.

Despite significant investigation, CDH-PH lacks a standard, consensus-based definition. Initial cardiac catheterization of

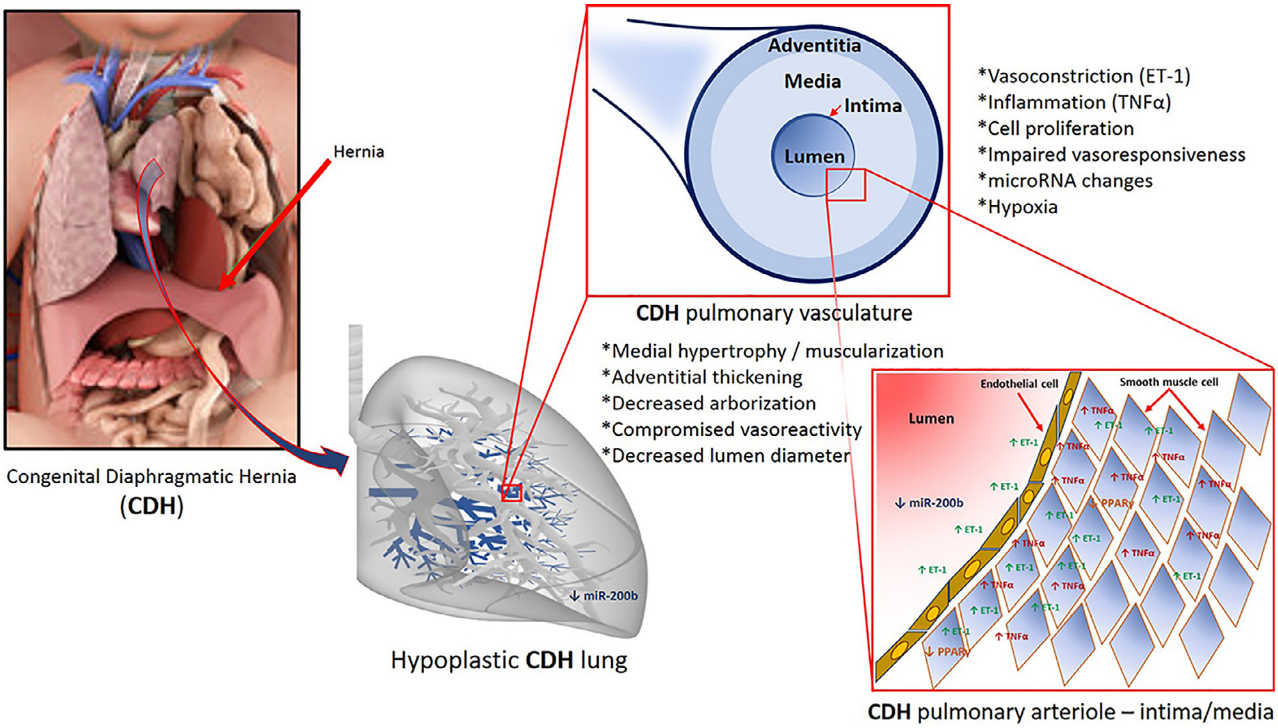

Fig. 1 – Overarching look at CDH-associated pulmonary hypertension including detailed changes at the cellular and subcellular levvels.

infants with CDH identified main PA pressures that almost always exceeded 25 mmHg.[42] Further, they found that a persistent R→L shunt, indicative of high pulmonary vascular resistance, portended a poor prognosis.[42] Determination of the ratio of the TR jet (estimation of the RVSP) to the SBP is an objective measurement used to estimate the PAP. A ratio <0.5 may be considered mild (or none), 0.5–0.67 moderate, and >0.67 severe CDH-PH.[43] Given the fact that survival is very high if a RVSP/SBP ratio is <0.5, this is often referred to as normal or no PH.[43,44] A ratio >1.0 (also called “systemic” or “supra-systemic” RVSPs) is also commonly used to define extreme CDH-PH and, particularly when measured beyond 3 weeks, portends a poor prognosis. In addition to the RVSP/SBP ratio and in cases where the TR jet cannot be measured, secondary signs of PH, including the intraventricular septum characteristics, RV anatomy and function, and PDA shunting (Table 2), can aid in the evaluation.[39]

Despite the frequent use of echocardiography in CDH patients, the optimal timing of evaluation of CDH-PH is unknown. Physiologic transitional pulmonary circulatory changes occur within minutes of birth but may persist for hours, days, or even weeks.[45,46] During these physiologic changes, echocardiographic RSVP measurements may be elevated without being pathologic. Conversely, early recognition of PH can help direct care and serve as a baseline for re-evaluation. Generally, an early (within 12 h of birth) evaluation is recommended. Repeated evaluation can be obtained based on patient trajectory and to monitor specific treatment response. Sometime around 3–4 weeks is also a reasonable time-point for prognostic evaluation.[43,44]

Evaluation and monitoring of pulmonary hypertension

Given the severity of the pathology and overall lack of effective treatment options, CDH mortality often occurs during the first days to weeks of life. However, advances in early diagnosis, surgical, and medical therapies have slowly, but steadily, improved overall survival in the last few decades.[47] Currently, in-hospital mortality is around 29%, and 1-year mortality rates range between 20–45%.[38] As mortality has improved, the need for long term monitoring of CDH-PH patients has become a burgeoning area of investigation. As noted above, right heart catheterization (RHC) is the gold standard for diagnosis of PH. However, RHC is invasive and

Table 2 – Typical echocardiographic findings indicative of CDH-PH.

Echocardiographic finding	Normal	PH
RVSP/SBP ratio (TR jet estimation)	<0.5 or unmeasurable	0.5–0.67 (moderate); 0.67–1.0 (severe); >1.0 (extreme)
Intraventricular septum (location)	Normal or relaxed toward the RV	Flattened or bowing or paradoxical
PDA shunt	Left to Right	Right to Left or bidirectional
RV wall	Normal thickness[88]	Hypertrophic / thickened
RV lumen	Normal size	Dilated / enlarged
RV function	Physiologic (systolic excursion)	Dysfunction (qualitative)

carries risks associated with general anesthesia, radiation exposure, and/or cardiopulmonary damage, especially in patients with already aberrant cardiopulmonary vascular development. For these reasons, repeated use of RHC for long-term follow-up of CDH-PH patients is not always safe or practical. Echocardiography has emerged as a more readily available, non-invasive study to mark the progression of CDH-PH in survivors. Multiple different flow markers have been proposed as prognostic for progression of CDH-PH; they include the tricuspid regurgitation jet peak velocity, patent ductus arteriosus (PDA) flow direction and velocity, ventricular septum configuration, and pulmonary regurgitation. These measures are limited given that echocardiograph measures are frequently inaccurate or unable to be obtained in pediatric patients. Additional study is needed on these measures as well as other echocardiography findings before determining which method is most appropriate for long-term follow up. Moreover, there are limited data on the appropriate frequency and duration with which to follow CDH-PH survivors. For patients discharged with PH, some centers obtain echocardiograms quarterly to semi-annually for the first few years of life or until resolution of signs of PH (and weaning from PH medications).[48] Some centers follow patients into adolescence, though there are no data to suggest that patients are at risk for exacerbation or return of PH once it has resolved. Despite this, PH may persist, sub-clinically into late childhood, so a long-term follow up strategy is important.[48]

Utility of noninvasive vascular compliance

Despite the limitations of echocardiographic measures, certain indices that have been validated in adults with PH are now being studied in infants with CDH-PH. The ratio of right ventricular (RV) systolic pressure using peak tricuspid valve regurgitation velocity (TRV) jet to the velocity time integral across the pulmonary valve outflow (VTIpv) on Doppler has correlated well with invasive measures of pulmonary vascular resistance.[37] In this ratio, TRV represents the transpulmonary gradient, and VTIpv measures RV cardiac output. Modifying for the Bernoulli equation yields the ratio of $TRV^2/VTIpv$. A retrospective review of echocardiograms performed in the first 48 hours of life of CDH infants revealed significantly higher TRV/VTIpv and $TRV^2/VTIpv$ in patients who died or required ECMO, compared to infants that survived without ECMO. The data also demonstrated that both TRV/VTIpv and $TRV^2/VTIpv$ have high sensitivities (92.3%, 96% respectively) and negative predictive values (89%, 90.9% respectively) for prediction of mortality or the need for ECMO. Further study is needed in larger populations of CDH infants to develop and validate these indices before their widespread use in CDH-PH.[37]

Right and left ventricular dysfunction

Despite the significant focus on the pulmonary vascular/parenchymal dysplasia and pulmonary vasculature pathophysiology in CDH, there has been increasing investigation on aberrant cardiac development and ventricular dysfunction in this population. Several studies using pre- and post-natal echocardiography in CDH infants have shown decreased biventricular systolic and diastolic function.[49] The exact developmental mechanism of these findings is not yet clear. It is likely that some component of the pathology, including left ventricular (LV) hypoplasia and increased LV strain, may be the result of external compression by abdominal viscera. This is supported by the fact that patients with right-sided CDH show less severe left atrial and ventricular pathology, while concomitantly developing worse right ventricular (RV) hypoplasia.[49] Some of the echocardiographic findings of LV dysfunction correct with surgical correction of the diaphragmatic defect, while others persist into adulthood.[50] While not the only contributing factor, it is likely that increased pulmonary vascular resistance contributes to right heart strain and decreased RV function.[50] Notably, while RV dysfunction may be the *result* of CDH-PH, LV dysfunction likely contributes to, exacerbates, and/or prevents resolution of CDH-PH. This topic is addressed completely in another manuscript in this edition of *Seminars*. The critical elements of the 3 cornerstones of pathophysiology in CDH, pulmonary hypertension, pulmonary hypoplasia, and ventricular dysfunction are shown in Fig. 2.

Biomarkers

Despite the numerous pathways implicated in CDH-PH, there is a paucity of data regarding the use of specific chemical biomarkers for prognostics or for long term follow-up. One study found that, CDH-PH infants with poor outcomes (death or need for respiratory support at 8 weeks) had significantly higher median plasma BNP levels on the first day of life, when compared to CDH-PH infants with good outcomes (survival and breathing room air at 8 weeks).[51] Another study of 13 CDH-PH patients found that elevated NT-proBNP, the pre-prohormone of BNP, is a sensitive, but not specific, predictor of mortality. These early results are promising, but larger, multi-center trials are necessary to standardize the significant cut-off values and optimal lab timing for these possible CDH-PH biomarkers.[51] As mentioned above, there is ongoing research into the role of micro-RNAs in the development of CDH-PH. While not yet formally investigated, there is some optimism that specific microRNAs will be useful as prognostic bioindicators in CDH infants.[30]

Imaging as a biomarker for long-term follow up

In addition to chemical biomarkers for PH in CDH survivors, there could be a role for following patients with serial imaging studies, such as echocardiography or lung perfusion scans. One longitudinal study followed CDH-PH survivors with repeat echocardiography and lung perfusion scans at 6 months to 1 year of age and again at 2–5 years of age. In a cohort of 24 CDH-PH infants, there was a significant improvement in PH from infancy, measured by right ventricular systemic pressure (RVsp) on echocardiography. In CDH infants, there is a decrease in blood flow to the affected lung, resulting in a “perfusion bias.” In follow-up lung perfusion scans at 2–5 years old, CDH-PH survivors showed a decrease in lung perfusion bias compared to infancy, though it rarely returned

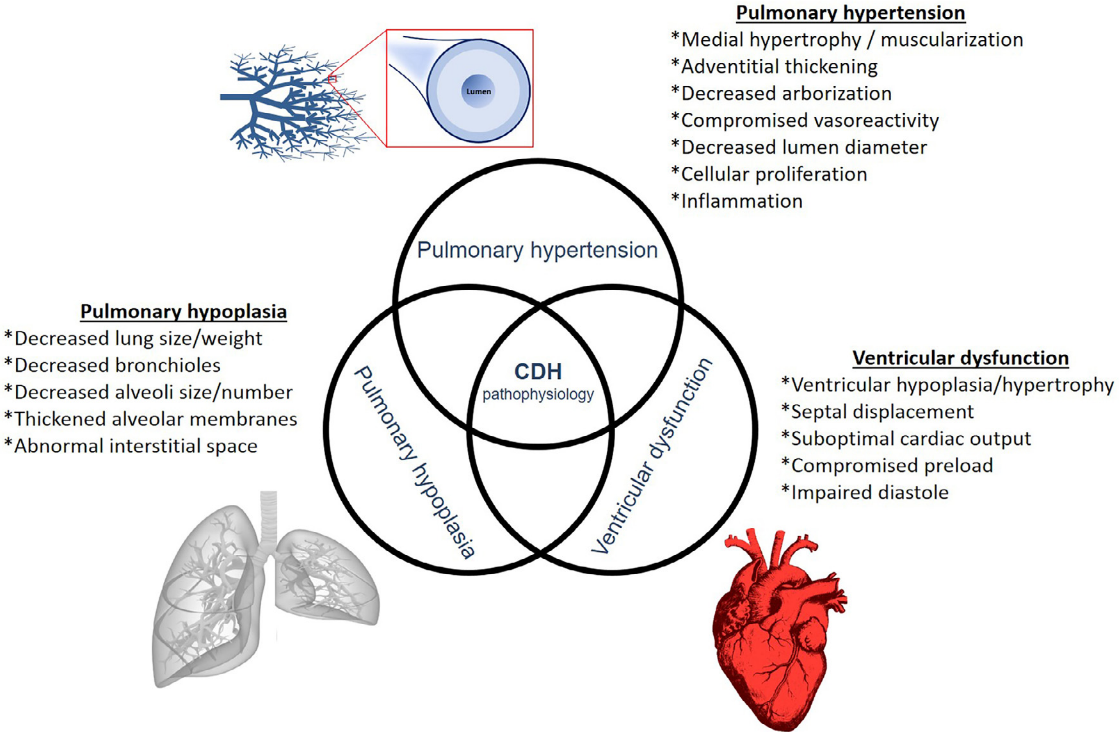

Fig. 2 – The interplay between the 3 cornerstones of pathophysiology in CDH: 1. Pulmonary hypertension, 2. Pulmonary hypoplasia, and 3. Ventricular dysfunction.

to normal levels.[52] Wilson and coworkers noted that ventilation-perfusion (V/Q) mismatch that progresses portends compromised activity adaptation and that significant V/Q mismatch (>1.2) was associated with larger defects, ECMO, and possibly CDH-PH.[53] While these data suggest good long-term outcomes for CDH survivors, additional studies are needed with larger populations and longer term follow-up, into young adulthood.

Natural history of CDH-PH

A retrospective review of infants with CDH shows that about 70% of patients will have elevated RSVP (>2/3 systemic systolic blood pressure), indicative of PH, on their initial echocardiogram.[17] Of these patients, 38% were placed on ECMO versus only 15% of patients without PH on the initial echocardiogram. Approximately 60% of patients had their RSVP normalize between their first and second echocardiograms, within the first few days to weeks of life. One study found 100% mortality in patients with echocardiograph evidence of PH (elevated RSVP) at 3 weeks, but there is more recent data that suggests better outcomes among patients with persistent PH.[44] A multi-institutional cohort study found that, when measured at 1 month, an RVSP/SBP ratio <0.5 was associated with a 98.6% survival, a ratio 0.5–0.67 was associated with a 92.6% survival, and a ratio >0.67 was associated with a 43.9% survival.[43] In summation, these data suggest that PH is strongly associated with CDH mortality, along with other outcomes.

Current management of CDH-PH

The complexity of CDH-PH continues to pose a challenge to pediatric intensivists and surgeons. Like most multifactorial diseases, CDH-PH requires a multimodal treatment strategy. Early diagnosis has allowed therapy to be started immediately at birth. Initially, avoiding fluid overload, optimizing blood pressure, and minimizing ventilator-associated lung injury are all key steps to limiting progression of CDH-PH. Oxygenation is a critical component of therapy. Oxygen acts as both a pulmonary vasodilator but also a source of free radicals at high concentrations.

Inhaled nitric oxide (iNO) is a common therapy for CDH-PH in many centers. Despite its widespread use, the data supporting efficacy in CDH-PH is lacking. NO is a vasodilator that can reduce ventilation-perfusion mismatching.[54] iNO is used in nearly 75% of infants with CDH-PH.[17] The AHA/ATS guideline notes that it can be used to improve oxygenation among infants with CDH and severe PH (though cautiously if LV dysfunction is suspected).[3] However, evidence has shown that it may not improve survival or decrease the need for ECMO. In 1997, the NINOS trial, a randomized, multi-center trial showed no difference in survival or need for ECMO among infants with CDH.[55] Subsequent clinical trials and studies using large CDH databases also failed to identify improvements in ECLS utilization or mortality with iNO use in CDH.[17,18]

Recent investigation suggests that the combination of iNO and milrinone can improve PH in CDH infants. Milrinone, a phosphodiesterase (PDE3) inhibitor that acts as a vasodilator and inotrope, has been shown to improve oxygenation in infants with persistent pulmonary hypertension of the newborn (PPHN). Although limited, studies of milrinone in CDH-PH demonstrated improved oxygenation index, decreased right ventricular pressure, and increased left ventricular ejection fraction. Additional multicenter, randomized trials are needed to identify the optimal use of milrinone in CDH-PH.[56]

Retrospective data on the administration of prostacyclin (epoprostinol *or* Flolan and treprostinil *or* Remodulin) and prostaglandin E1 (alprostadil) shows that they are well tolerated and can improve both echocardiographic markers of PH and serum BNP levels. PGE is a potent pulmonary vasodilator known to decrease pulmonary artery pressure; it also maintains patency of the ductus arteriosus, which decreases right ventricular strain. Infants with CDH who have persistently high (usually suprasystemic) right ventricular systolic pressures may benefit from the addition of a prostaglandin.[3,57,58] While more study is needed, current American Heart Association Guidelines recommend the use of PGE in infants with CDH-PH.[59]

Treprostinil improves PH

Treprostinil is one of several synthetic prostacyclin analogs; it is a vasodilator currently approved for treatment of pulmonary arterial hypertension in adults. Recent retrospective data, along with a number of case series, have shown that treprostinil is well tolerated in refractory PH in CDH infants. A review of 17 patients with moderate to severe CDH-PH treated with treprostinil demonstrated improvement in markers of PH including BNP and RVSP to SBP ratio as measured on echocardiogram.[60] The studied cohort showed significantly decreased PH severity after treatment with no change in mortality and no adverse events related to treprostinil administration. Given the small sample size and retrospective nature of the current literature, bigger, prospective models are needed to assess the optimal dosing, population, and timing of treprostinil administration for CDH-PH.[60]

Endothelin receptor antagonists

Endothelin receptor antagonists block ET_A, ET_B, or both receptors. This leads to vascular smooth muscle relaxation. Bosentan (Tracleer) is a dual endothelin receptor antagonist that has been used to treat CDH-PH.[41] A limited number of studies have shown that bosentan therapy is well tolerated in infants with CDH and may decrease pulmonary arterial pressure compared to placebo.[61]

Sildenafil

Sildenafil is a phosphodiesterase-5 (PDE-5) inhibitor that has recently gained more traction as a potential treatment for CDH-PH. Sildenafil leads to pulmonary vasodilation by potentiating the effects of nitric oxide via a cGMP-dependent mechanism.[62] A summary of current pharmacologic therapies targeting CDH-PH is shown in Table 3.

Moreover, sildenafil has been demonstrated to improve the pulmonary vasculature in CDH rodent models. The nitrofen-rat model closely models human CDH-PH pathophysiology. CDH is associated with a decrease in total pulmonary vascular development, reduced vascular branching, and increased vascular smooth muscle thickening. Data suggests that antenatal administration of sildenafil during lung development significantly reduces vascular smooth muscle thickening. Additionally, CDH lungs treated with antenatal sildenafil showed decreased aberrant perivascular cell differentiation, compared to non-treated CDH lungs. This suggests that, with further study, antenatal sildenafil could be a viable treatment strategy for human CDH-PH.[63]

Unfortunately, formal, randomized studies on the use of sildenafil in CDH-PH are not yet available. However, retrospective reviews of continuous IV sildenafil in infants with CDH suggest that this therapy is safe and can lead to improved oxygenation.[64] While early initiation of sildenafil (antenatal, first day of life, etc.) may benefit patients with CDH-PH additional investigation is needed to establish dosing, timing, and long-term outcomes.

For patients that are unresponsive to optimal ventilator support, fluid/blood pressure management, and pharmacologic treatment, extracorporeal life support (ECLS or ECMO) is the next step in most management algorithms.[3,62] This strategy is discussed elsewhere in this issue of *Seminars*.

Long term PH follow-up

Increased survival of CDH-PH infants has led to questions about the most appropriate methodology and frequency with which to perform long term follow-up. Only a handful of studies, all with different methodology and relatively small sample sizes, have examined the necessity of CDH-PH follow up. Almost universally, follow-up was done using echocardiographic measures of PH. Based on the small amount of prospective data available, only a minority (<10%) of CDH-PH patients have persistent PH at 6 and 12 months.[65] These patients required longer duration of mechanical ventilation and hospital stays than CDH-PH survivors without persistent PH. They also required more intense vasodilator therapy at the time of diagnosis. This suggests that echocardiographic follow-up of CDH-PH infants should be reserved patients with more severe initial disease. However, larger, multicenter investigation is needed before widely-accepted guidelines can be made.

Future therapies for PH

Given the complexity of CDH-PH and the lack of effective treatment regimens, ongoing discovery of new therapies is critical to improving outcomes. In addition to novel therapies, there is ongoing research into optimizing the currently existing approaches to PH in general. Many of the therapies discussed below are still in pre-clinical and early clinical trials.

Already in phase III trials for adult PH, Selexipag is an oral prostacyclin receptor agonist that is a highly selective vasodilator.[66] A recent phase III trial found that Macitentan, a dual endothelin receptor antagonist (ERA) with sustained receptor binding and strong tissue penetration, reduced morbidity and mortality compared to placebo.[67] Riociguat, a soluble guanylate

Table 3 – Current therapies for CDH-PH.

Name	Mechanism of action	Effect	Dosing	Refs
Milrinone	Phosphodiesterase-3 inhibitor	Improve RV function and relax the PA	0.25–0.75 μg/kg/min	61
Sildenafil (IV)	Phosphodiesterase-5 inhibitor	Decrease PA pressure, decrease PVR, improve CI	100–290 μg/kg/hr	89
Sildenafil (PO)	Phosphodiesterase-5 inhibitor	Decrease PA pressure, decrease PVR, improve CI	0.3–2.0 mg/kg/dose Q 6–8 hr	61,90
Bosentan	Endothelin receptor A/B antagonist	Vasorelaxation	1–2 mg/kg BID	61
Prostaglandin E_1 Alprostadil	Hormone – smooth muscle relaxation	Vasodilation and maintenance of the PDA	0.05–0.20 μg/kg/min	91
Epoprostenol (Flolan®)	Synthetic prostacyclin - smooth muscle relaxation	Vasodilation	2–10 ng/kg/min titrate 0.5–2 ng/kg/min	92
Treprostinil (Remodulin®)	Synthetic prostacyclin - smooth muscle relaxation	Vasodilation	1.25–10 ng/kg/min titrate 0.5–2 ng/kg/min	92

cyclase stimulator that restores the nitric oxide-cGMP pathway, has shown promise in several phase III clinical trials, both as a first-line therapy and when used in an adjunct/rescue strategy.[68,69] Pathways without significant previous investigation that are theoretically promising include redox-dependent signaling (key in hypoxia-induced PH) and ion channelopathies (potassium and calcium channels are associated with vasomotor control in the pulmonary circulation).[66] Studies focused on the neonatal, infant, or pediatric population are lacking.

As previously discussed, the retinoic pathway has been implicated in CDH development. Studies on maternal intake of vitamin A (retinol) suggested that lower daily intake of vitamin A during gestation may increase the risk of CDH.[11] Compared to controls, CDH is strongly associated with both low retinol and retinol binding protein levels among newborn infants, and there is some data that prenatal administration of retinoic acid may attenuate CDH-PH.[70,71]

Given what has been described as “fixed” pulmonary hypertension among patients with CDH, strategies that hold the promise of *reversing* the pulmonary vascular remodeling that occurs in CDH-PH deserve focused investigation. Anti-proliferative strategies, including tyrosine kinase inhibitors such as Nilotinib, have shown early, preclinical promise in reversing vascular remodeling in PH.[72] Alteration of transcription factor signaling, including peroxisome proliferator-activated receptor (PPAR) factors and Notch signaling inhibitors have shown the potential to downregulate cellular proliferation.[73,74] Anti-inflammatory strategies, such as inhibiting stromal-derived factor-1 (SDF-1), can mitigate ongoing pulmonary vascular inflammation, minimizing ongoing vascular remodeling.[75]

Stem cell and progenitor cell therapy have also been investigated as a potential treatment for CDH and PH. Pre-clinical models of PH have shown that mesenchymal stromal cells (MSCs) can reverse the cardiovascular effects of PH. Extracellular vesicles, also known as exosomes, are small lipid membrane vesicles that are released into the extracellular environment. They have highly variable content and are mediators of exocrine and paracrine cell function, allowing for cellular exchange of proteins, cytokines, nucleic acids, and growth factors. MSC-derived extracellular vesicles (MSCEv) have been shown to hasten recovery and mediate pulmonary parenchymal restoration following ventilator-induced lung injury.[76,77] Sources of MSCs include the bone marrow, umbilical cord, cord blood, or amniotic fluid.[78–80] In the nitrofen-rodent model of CDH, pre-natal infusion of MSCs yielded CDH-pups with decreased pulmonary arterial medial thickening and improved alveolar morphology compared to controls.[81] In adults with PH, the use of intravenous endothelial progenitor cells, transfected with endothelial nitric oxide synthase, has been shown to be safe and to improve short-term hemodynamics and walking distance over 6 months.[82]

Monocyte chemoattractant protein-1 (MCP-1) is a chemokine shown to have increased levels in patients with PH. It is produced by multiple pulmonary cell lines including the alveolar epithelium, endothelial cells, and smooth muscle cells. Increased expression of MCP-1 activates perivascular monocytes and macrophages to produce a pro-inflammatory phenotype in the pulmonary vasculature that contributes the vascular remodeling seen in PH. Additionally, the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) has been shown to play an important role in fetal lung and pulmonary vasculature development. PPARγ signaling triggers an antiproliferative pathway that, when disrupted, leads to the aberrant SMC proliferation seen in PH. Activation of the PPARγ signaling pathway can lead to normal vasculogenesis and limit pathologic remodeling. Preliminary studies on the PPARγ-agonist rosiglitazone (thiazolidinedione) indicate that it can inhibit overexpression of MCP-1. Studies in the nitrofen-rat model of CDH-PH show that prenatal administration of rosiglitazone activates the PPARγ signaling pathway and significantly decreases perivascular MCP-1 expression. This resulted in significant improvement in pulmonary vessel thickness as well as decreased numbers of pulmonary monocytes/macrophages compared to placebo-treated CDH-rodents. Next steps include investigations of alterations in MCP-1 and PPARγ expression in human CDH pulmonary vasculature and then investigation into the therapeutic use of rosiglitazone.[83]

ECMO is a commonly used strategy in CDH-PH to improve oxygenation. Some centers have investigated the use of paracorporeal lung assist devices (PLADs) sometimes referred to as implantable or artificial lungs. Limited study in animal models of PH and case reports of PLAD in infancy suggest that PLAD can improve oxygenation in PH. It is primarily described as a bridge to lung transplantation. As of yet, the role of PLAD specifically in CDH-PH is undescribed, especially as transplantation in CDH also needs further investigation. One potential benefit of PLAD, in comparison to ECMO, is that it may allow extubation and early ambulation and physical rehabilitation for patients.[84]

In addition to the artificial lung, there have been investigations into the utility of an "artificial placenta."[85–87] The use of arteriovenous ECMO via umbilical vessels to provide extrauterine support in animal models has shown promise in improving fetal lung development. While not yet specifically tested in CDH-PH models, the artificial placenta system showed increased production of surfactant, increased lung weight, and increased mature type II pneumocytes.

Conclusion

CDH is a complex anatomic and histologic condition of the newborn. Despite many advances in diagnosis and therapy, it continues to have high morbidity and mortality. CDH-PH plays a central role in the overarching pathophysiology of CDH. A handful of treatment strategies and algorithms have been applied to infants with CDH-PH, but they all remain under-studied. There are a number of the new therapies for PH that are still under investigation with early clinical trials or pre-clinical models. Given that CDH-PH is a multi-factorial and multi-systemic entity, a combination of basic, translational, and clinical science is needed to change the management paradigm of this disease.

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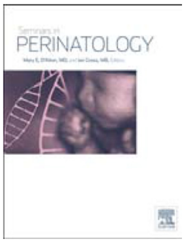

Congenital diaphragmatic hernia-associated cardiac dysfunction

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ABSTRACT

There is increasing evidence that cardiac dysfunction is a key contributor to CDH pathophysiology. Dysfunction in both right and left ventricles is common in the early neonatal period, contributes to clinical disease severity, and is associated with adverse outcomes including death and ECMO use. Early and routine assessment of ventricular function and pulmonary artery pressure may guide individualized clinical decision-making, including use of pulmonary vasodilators, cardiotropes, ECMO, and timing of surgical repair. Minimizing cardiac dysfunction, whether by prenatal, postnatal or perinatal treatment strategies, may lead to improved outcome in CDH.

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Introduction

Congenital diaphragmatic hernia (CDH) remains a major cause of morbidity and mortality in the neonatal period and beyond.[1] Herniation of the abdominal contents into the fetal thorax is associated with hypoplasia of both lungs and developmental abnormalities of the pulmonary vasculature. The latter causes fixed and dynamic elevation of pulmonary vascular resistance (PVR) and pulmonary hypertension (PH).[2] Pulmonary hypoplasia and PH are considered the two key pathophysiologies in CDH, therefore treatment strategies have traditionally focussed on lung-protective ventilation and optimising PVR.[3,4]

Cardiac dysfunction is emerging as a third and equally important component of CDH pathophysiology.[5] Recent advances in functional echocardiography, combined with observational studies in the fetus and newborn have revealed the mechanisms and clinical significance of cardiac function in CDH.[6,7] This review aims to summarize the evidence for cardiac dysfunction as a key mediator of CDH severity and makes recommendations for clinical assessment and management.

Pathophysiology of ventricular dysfunction in CDH

Understanding cardiac function in CDH requires consideration of the interdependent function of both right and left ventricles, during diastole and systole, and the effects of changing loading conditions associated both with the underlying disease and the transition from fetus to neonate.

Abbreviations: CDH, congenital diaphragmatic hernia; ECMO, extra-corporeal membrane oxygenation; LV, left ventricle; PAP, pulmonary artery pressure; PDA, patent ductus arteriosus; PGE_1, prostaglandin E_1; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RV, right ventricle; STE, speckle tracking echocardiography

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The fetal heart in CDH

Fetal left heart structures appear to be smaller in CDH, regardless of the side of the diaphragmatic defect.[8,9] The extent of LV hypoplasia correlates with fetal lung hypoplasia.[10,11] Systolic function appears to be preserved in the fetus, but LV diastolic relaxation may be impaired.[12,13] The precise mechanisms of fetal LV hypoplasia are not fully understood, but may include primary disease-related alterations of embryological cardiac development as well as direct mechanical compression by herniated abdominal contents, together with reduced LV filling due to reduced pulmonary blood flow, and mediastinal shift leading to altered streaming of ductus venosus flow away from the left heart.[14,15]

Postnatal cardiac function in CDH

CDH is associated with failure of normal cardio-respiratory transition at birth. Structural and functional abnormalities of the pulmonary vasculature lead to persistent elevation of PVR and PAP for weeks or months after birth.[16–18] Chronic elevation of PVR promotes hypoxemic right-to-left shunting between the atria and across the patent ductus, and increases afterload on the right ventricle (RV) leading to changes in RV structure and function.

The postnatal RV in CDH

In the face of increased afterload the normally thin-walled RV dilates and hypertrophies, displacing the interventricular septum leftwards. Hypertrophy, due to myocardial hyperplasia, pre-disposes the ventricle to sub-acute ischaemia.[19]

Structural changes in the RV are accompanied by RV dysfunction, which has been observed within the first 48 h after birth.[20,21] RV dysfunction in CDH, as in other pulmonary hypertensive diseases, predominantly affects ventricular relaxation in early diastole.[22]

RV diastolic dysfunction may persist throughout the neonatal period, in conjunction with ongoing elevation of PVR and PAP, exacerbated at times of surgery, infection, and weaning of sedation.[20,23] Patterns of PAP and RV function beyond the neonatal period remain uncertain. Echocardiographic evidence of PH may resolve within the first year of life, but RV dysfunction has been detected at up to 5 years of age.[24,25]

The postnatal LV in CDH

LV dysfunction is also common in CDH.[5] Recent studies using Speckle Tracking Echocardiography (STE) have demonstrated global systolic and diastolic LV dysfunction as well as abnormal synchrony of myocardial regions, associated with reduced left ventricular output.[26–28]

LV dysfunction in CDH is probably multi-factorial, as illustrated in Fig. 1. Dysfunction in the RV will lead to secondary dysfunction in the LV via mechanisms of ventricular interdependence including shared muscle fibers, pericardial space and septum.[29,30] Displacement of the septum physically restricts LV filling, impairing diastolic function.[31,32]

Primary LV dysfunction has also been observed in the absence of RV dysfunction and affecting myocardial regions in the free wall of the LV.[27] Primary LV dysfunction may be due to a "perfect storm" of pathological factors in the transitional period: First, reduced pulmonary blood flow and LV preload; second, LV hypoplasia; third, the acute increase in LV afterload at birth; and fourth, the negative effects of systemic hypoxia and acidosis (Fig. 1).

Early LV dysfunction in CDH appears to be transient, typically improving within the first days of life.[5,33] Importantly,

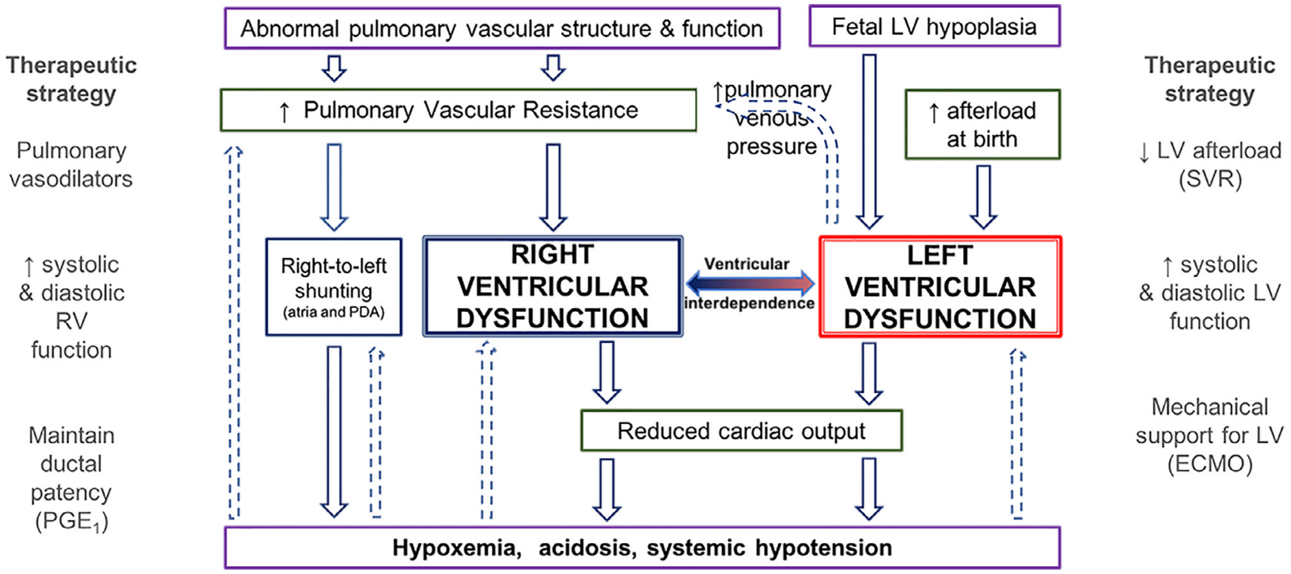
Therapeutic strategy
Pulmonary vasodilators
↑ systolic & diastolic RV function
Maintain ductal patency (PGE_1)
Abnormal pulmonary vascular structure & function
Fetal LV hypoplasia
↑ Pulmonary Vascular Resistance
↑pulmonary venous pressure
↑ afterload at birth
Right-to-left shunting (atria and PDA)
RIGHT VENTRICULAR DYSFUNCTION
Ventricular interdependence
LEFT VENTRICULAR DYSFUNCTION
Reduced cardiac output
Hypoxemia, acidosis, systemic hypotension
Therapeutic strategy
↓ LV afterload (SVR)
↑ systolic & diastolic LV function
Mechanical support for LV (ECMO)

Fig. 1 – Mechanisms of ventricular dysfunction in CDH.
RV, right ventricle; LV, left ventricle; SVR, systemic vascular resistance; PGE_1, prostaglandin E_1; ECMO, extra-corporeal membrane oxygenation.

LV dysfunction may lead to increased pressure in the pulmonary veins, contributing to increased PVR independently of the structural abnormalities in the pulmonary vasculature.

Cardiac dysfunction, clinical presentation and relationship to outcome

Cardiac dysfunction is a key contributor to the clinical presentation in severe CDH. Biventricular dysfunction leads to reduced cardiac output and promotes right-to-left shunting across the patent arterial duct (PDA) giving rise to systemic hypotension, acidosis, and hypoxemia. These in turn exacerbate cardiac dysfunction and pulmonary vasoconstriction leading to spiralling clinical deterioration.

The relationship between cardiac dysfunction and outcome in CDH has been revealed in smaller retrospective studies, and recently confirmed in large multi-centre registry analysis [**Patel et al., in submission**]. Early RV myocardial dysfunction is associated with increased mortality and use of extra-corporeal membrane oxygenation (ECMO).[20,34,35] Similarly, reduced early LV strain, a measure of systolic function measured by STE, is associated with death and ECMO use, and correlates with length of stay and duration of respiratory support in survivors.[26,36] Left ventricular output is also lower in CDH cases who require ECMO.[37]

Early LV systolic function correlates with prenatal lung volumes, measured by MR imaging.[26] However, cardiac dysfunction can also occur in infants with small diaphragmatic defects and limited respiratory compromise.

Principles of CDH management

Based on current evidence, we propose three guiding principles for clinical management in CDH:

(1) Consider the relative contributions of cardiac dysfunction, elevated PVR, and pulmonary hypoplasia to clinical status

(2) Early and regular echocardiographic assessment of cardiac function and pulmonary artery pressure

(3) Targeted, individualized treatment based on clinical and echocardiographic findings

Assessment of cardiac function in CDH

Echocardiographic assessment should be performed in all cases of CDH to identify any structural cardiac disease and assess ventricular function, dimensions, and PAP/PVR.[38] Functional echocardiography should combine qualitative and quantitative techniques in accordance with current international guidelines, Table 1.[39–41] Assessment should be performed by an experienced operator, taking into consideration the technical challenges of mediastinal shift and clinical stability in CDH patients.

Functional assessment should be performed within the first 4–12 h of life, after the initial transitional period and in anticipation of potential early cardiac dysfunction. Repeat assessment should be performed regularly thereafter to guide decision-making in the pre- and post-operative periods, during weaning of support, and at times of unexpected deterioration. Pre-discharge assessment helps to determine the need for ongoing cardiovascular therapy and follow-up.[24,42]

Kinsella and colleagues, have additionally proposed routine fetal evaluation of cardiac dimensions.[43] Whether fetal ventricular size can predict post-natal dysfunction and outcome is an area of ongoing investigation.

Use of postnatal cardiac function assessment to guide targeted therapy

Right ventricular dysfunction

RV dysfunction, in combination with clinically significant PH (elevated PAP/PVR with hypoxemic right-to-left shunting) is an indication for therapies to reduce PVR and RV afterload. Sedation, acid-base status, and lung recruitment should be optimized, followed by structured, targeted use of pulmonary vasodilators.[44]

Inhaled nitric oxide and sildenafil, a phospho-diesterase 5 inhibitor, augment pulmonary vasodilating action of endogenous nitric oxide and are associated with improved oxygenation in CDH.[45] However, response to these vasodilators is variable and recent, retrospective, multi-institutional clinical investigations have shown that iNO is unlikely to decrease the need for ECMO or improve mortality.[46–48] One possible explanation is that pulmonary vasodilators may exacerbate pre-existing LV dysfunction in CDH by increasing pulmonary venous return to the failing LV.[3,39,49] An ongoing randomized controlled trial of inhaled nitric oxide versus intravenous sildenafil in CDH may address these uncertainties.[50]

Milrinone, a phospho-diestarase 3 inhibitor, is in theory ideally suited for CDH. As well as promoting pulmonary vasodilatation by targeting the prostacyclin pathway, milrinone directly augments ventricular function.[51] Milrinone is widely used in CDH management and an RCT of early use is in progress.[52]

Prostaglandin E1 (PGE1) may also be used to maintain the patent ductus as a “blow-off” valve for the pressure-loaded RV and augment systemic blood flow, as illustrated in Fig. 2.[53] Our practice is to use PGE 1 early if the following criteria are met: i) the RV is dilated and dysfunctional, ii) PAP $\geq$ systemic blood pressure (bidirectional or right-to-left PDA shunt) and iii) the duct is closing or closed.

Left ventricular dysfunction

In the presence of clinically significant LV dysfunction, associated with reduced cardiac output and systemic hypotension, myocardial function should be supported, using inotropic agents such as dobutamine or low dose epinephrine. Routine administration of volume, without evidence of hypovolaemia, is questionable. Our practice is to target a systemic blood pressure in the upper-normal range. Vasopressors should be used with caution, they may exacerbate LV

Table 1 – Echocardiographic techniques for assessment of pulmonary artery pressure and cardiac function in congenital diaphragmatic hernia.

Parameter	Technique	Notes and limitations
Pulmonary artery pressure assessment		
Peak Tricuspid Regurgitation Velocity (TR_{max})	Estimates RV peak systolic pressure using modified Bernoulli equation ($\text{RVSP} = 4(\text{TR}_{\text{max}})^2$)	TR_{max} may be absent or difficult to measure accurately.
Patent arterial duct (PDA) flow	Doppler assessment of direction & velocity, estimates PAP relative to systemic BP	Requires patent ductus. Qualitative assessment of PAP.
Interventricular shape and position	Indirect assessment of right ventricular pressure and PAP	Qualitative assessment only.
Acceleration time: right ventricular ejection time ratio (AT:RVET)	Time intervals measured from Doppler of RV outflow.	Correlates with pulmonary vascular resistance. Does not quantify PAP.
Cardiac function assessment		
“Eyeball” of function from 2D loop	Subjective assessment of function from 2D images in long and short axes	Subjective, qualitative, high inter-observer variability
Ejection Fraction (EF)	Percentage of change in LV volume from end- diastole to end-systole	Angle- and load-dependent, inter-observer variability, affected by septal shape and dysfunction.
RV Fractional Area Change (FAC)	Percentage change in RV area between end-diastole and end-systole	Load dependent, high inter-observer variability. Global measure of function.
Tricuspid Annular Systolic Excursion (TAPSE)	Longitudinal displacement of the lateral tricuspid valve annulus during systole	Highly load- and angle-dependence. Assesses systolic function only.
Atrio-ventricular valve (AV) inflow	Doppler analysis of diastolic inflow to ventricles	Diastole only, highly load-dependent.
Right and Left Ventricular Outflow (RVO and LVO)	Estimation of ventricular output, product of stroke volume and valve area	Time-consuming, poor repeatability, affected by shunts
Myocardial Performance Index (MPI)	Global measure derived from time intervals	Highly load-dependent, does not distinguish systolic / diastolic function
Systolic:Diastolic duration (SD:DD)	Time intervals obtained from outflow Doppler.	Heart-rate and load-dependent. Does not distinguish systolic and diastolic function
Tissue Doppler Imaging (TDI) of myocardial velocities	Longitudinal systolic and diastolic velocities measured in basal myocardium of RV, LV and septum.	Quantitative assessment of function. Angle- and load-dependent.
Ventricular strain assessed by Speckle Tracking Echocardiography (STE)	Quantitative assessment of global & regional deformation (strain, strain rate, twist) in multiples planes.	Specific hardware, software, user experience and optimal images. Inter-vendor differences.

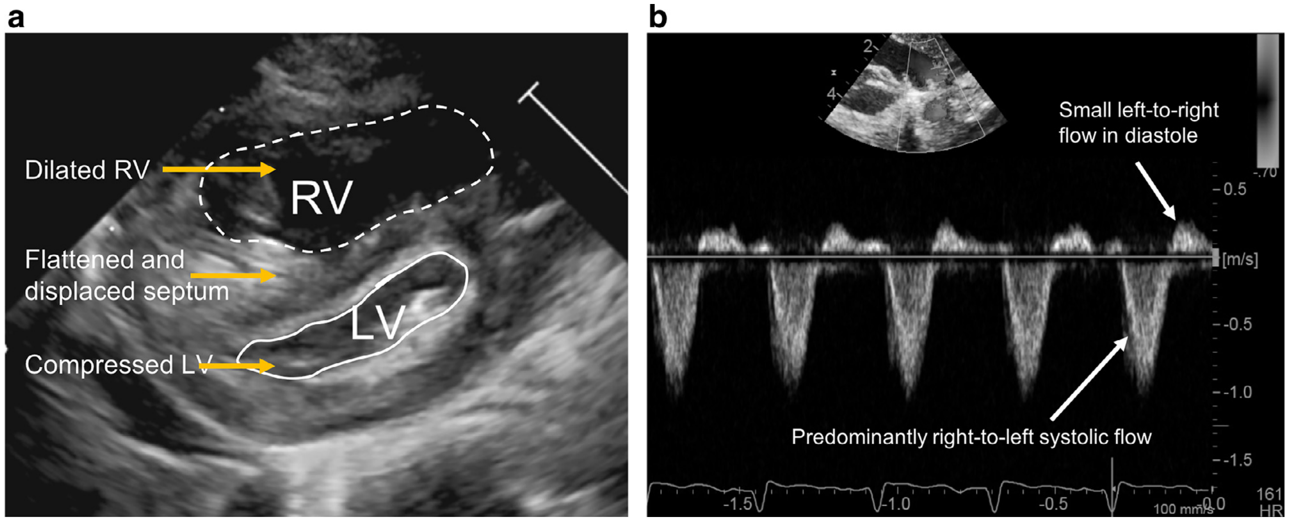
a
Dilated RV
RV
Flattened and displaced septum
LV
Compressed LV
b
Small left-to-right flow in diastole
-0.5
[m/s]
-0.5
-1.0
-1.5
Predominantly right-to-left systolic flow
-1.5
-1.0
-0.5
100 mm/s
0.0
161 HR

Fig. 2 – Right ventricular dysfunction in CDH.
Prenatally diagnosed left sided CDH. Prenatal observed-to-expected total fetal lung volumes (TFLV) 25%. Severe hypoxemia and acidosis associated with severe RV dilatation and dysfunction, in conjunction with a constricting arterial duct (Fig. 2a, short axis view demonstrating dilated RV, flattening of interventricular septum and compression of LV). Pulmonary vasodilator therapy (inhaled NO and IV sildenafil) commenced with PGE$_1$ to maintain ductal patency (Fig. 2b, Doppler waveform of predominantly right-to-left flow through patent arterial duct). RV function and clinical status improved over next 24 h, avoiding ECMO therapy.

afterload. If LV function is preserved, pressors such as vasopressin and noradrenaline may have a beneficial effect on pulmonary vascular tone and hemodynamics.[54–56]

Cardiac function and extra-corporeal membrane oxygenation (ECMO) use

ECMO use in CDH remains controversial.[57] Understanding the relative contribution of cardiac dysfunction to clinical disease severity can guide rational ECMO use. In the setting of severe LV dysfunction, resistant to optimized medical therapy, ECMO can provide time for function to improve, prior to decannulation and CDH repair, as illustrated in Fig. 3.

Cardiac function and timing of surgical repair

Current international recommendations advocate surgical repair based on physiological criteria of oxygenation, blood pressure and acid-base status.[44,58] Cardiac function is a key determinant of these clinical parameters and should be incorporated into decision-making. Repair may be reasonably delayed until cardiac function has optimised.[5,20] However, Tanaka et al. have also reported improvement in LV function following early repair on the first day of life.[33]

Future investigation and therapeutic approaches

The relationship between cardiac function and outcome in CDH has now been confirmed in larger registry-based studies [**Patel et al., in submission**]. The results of prospective trials are still awaited to determine whether treatment of cardiac dysfunction can improve clinical outcomes.

New therapeutic approaches have potential to reduce cardiac dysfunction in CDH. Intact cord resuscitation, incorporating lung recruitment prior to delayed umbilical cord clamping, may be associated with improved pulmonary blood flow, LV function, and cardiac output.[59,60] Fetal drug therapies which modulate pulmonary vascular development may potentially reduce postnatal PVR and in turn improve RV and LV performance.[61,62]

Conclusion

Cardiac dysfunction is an important component of CDH pathophysiology and determinant of outcome. Early and regular assessment of cardiac function is an essential in clinical CDH management, to understand underlying pathophysiology and guide targeted, individualized treatment. Improved assessment, treatment, and prevention of cardiac dysfunction in CDH may lead to improved outcomes in this challenging condition.

Sources of support

None.

Disclosures

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

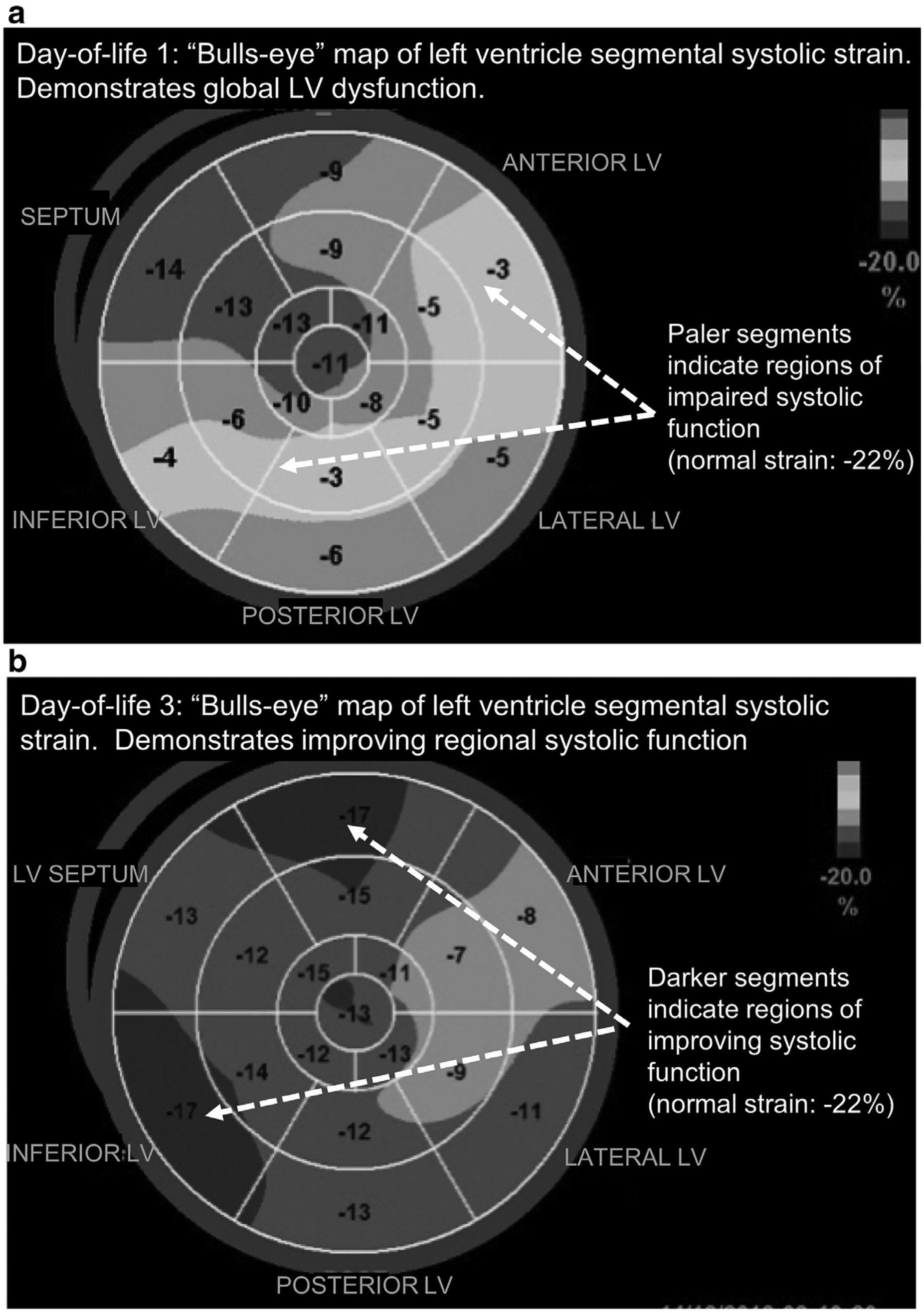
a
Day-of-life 1: “Bulls-eye” map of left ventricle segmental systolic strain. Demonstrates global LV dysfunction.
SEPTUM
ANTERIOR LV
INFERIOR LV
LATERAL LV
POSTERIOR LV
-9
-14
-9
-3
-13
-13
-11
-5
-11
-10
-8
-6
-5
-4
-3
-5
-6
-20.0
%
Paler segments indicate regions of impaired systolic function
(normal strain: -22%)
b
Day-of-life 3: “Bulls-eye” map of left ventricle segmental systolic strain. Demonstrates improving regional systolic function
LV SEPTUM
ANTERIOR LV
INFERIOR LV
LATERAL LV
POSTERIOR LV
-17
-15
-13
-8
-12
-15
-11
-7
-13
-12
-13
-14
-9
-17
-11
-12
-13
-20.0
%
Darker segments indicate regions of improving systolic function
(normal strain: -22%)

Fig. 3 – Left ventricular dysfunction in CDH.
Prenatally diagnosed left-sided CDH. Prenatal TFLV 28%. Severe hypoxemia, hypotension and acidosis associated with severe LV dysfunction on day 1 of life (Fig. 3a, right panel “Bull’s Eye” map of LV longitudinal systolic strain derived by STE. Paler segments represent regions of reduced systolic function). Patient received low dose epinephrine and milrinone and progressed to veno-arterial ECMO at 24 h of age. LV function improved rapidly by day 3 of life (Fig. 3b, left panel demonstrating improvement in global LV function), decannulated from ECMO day 4, patch repair performed day 5.

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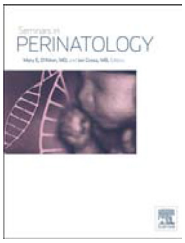

The influence of genetics in congenital diaphragmatic hernia

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ABSTRACT

Congenital diaphragmatic hernia (CDH) is a common birth defect that is associated with significant morbidity and mortality, especially when associated with additional congenital anomalies. Both environmental and genetic factors are thought to contribute to CDH. The genetic contributions to CDH are highly heterogeneous and incompletely defined. No one genetic cause accounts for more than 1–2% of CDH cases. In this review, we summarize the known genetic causes of CDH from chromosomal anomalies to individual genes. Both *de novo* and inherited variants contribute to CDH. Genes causing CDH are increasingly identified from animal models and from genomic strategies including exome and genome sequencing in humans. CDH genes are often transcription factors, genes involved in cell migration or the components of extracellular matrix. We provide clinical genetic testing strategies in the clinical evaluation that can identify a genetic cause in up to ~30% of patients with non-isolated CDH and can be useful to refine prognosis, identify associated medical and neurodevelopmental issues to address, and inform family planning options.

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Introduction

Congenital diaphragmatic hernia (CDH) is a common and severe birth defect that affects around 1 in 3000 live births.[1] It is characterized by incomplete formation of the diaphragm, leading to the herniation of the abdominal viscera into the chest cavity. CDH can occur as an isolated defect but ~40% of cases are non-isolated and associated with additional anomalies in other systems, most commonly including heart, brain, renal and genitourinary malformations.[1,2] Newborns with CDH often have severe respiratory distress resulting from pulmonary hypoplasia and pulmonary hypertension, which are the most significant causes of morbidity and mortality of CDH patients. Today, over 70% of cases can be prenatally diagnosed based on the imaging techniques including ultrasonography and fetal magnetic resonance imaging (MRI) at a mean gestational age of 24 weeks.[3,4] Prenatal diagnosis has altered the neonatal outcomes and significant progress has been made in the postnatal care of patients with CDH, with mortality rates reduced to ~30%.[5] However, the long-term morbidity and the medical cost for caring for CDH survivors remains a significant burden. Understanding the etiology of CDH is essential to discovery of new therapies to improve outcomes.

Both environmental and genetic factors contribute CDH. It has been known that nitrofen and manipulating retinoids cause CDH in animal models.[6,7] Most human cases are unexplained by known environmental factors. Evidence from

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genetic studies in patients with CDH and animal models provides important insight into the molecular mechanisms of diaphragm development. The genetic etiologies of CDH are highly heterogeneous. Chromosomal anomalies of aneuploidies, structural rearrangements, copy number variants (CNVs) and single-gene mutations all contribute to CDH. Identifying a genetic etiology for CDH in patients is increasingly clinically relevant to provide accurate prognostic information and guide medical management. Studies indicate that chromosomal anomalies detectable by karyotype are observed in 10% of CDH cases[8,9] and 3–10% of CDH are associated with a syndromic diagnosis.[8,10] Recent massively parallel sequencing techniques demonstrate that 10–22% of CDH patients have *de novo* damaging variants that are associated with a high relative risk of developmental disorders.[11–13] In this review, we summarize the current knowledge of CDH genetics.

Chromosomal anomalies associated with CDH

Chromosomal anomalies have been reported in about 10% of all individuals with CDH[1] and include complete or mosaic aneuploidies, cytogenetic rearrangements detectable by karyotype, and copy number variants (CNVs) identified by chromosome microarrays or other methods. Structural anomalies involve almost all chromosomes,[10,14–16] and an updated list of CDH associated chromosomal anomalies (Supplementary Table 1) and recurrent CNVs are provided (Fig. 1).

Aneuploidies

Aneuploidy is an abnormal number of chromosomes. CDH is observed infrequently with many aneuploidies including trisomies 13, 18 and 21 and rarely with Turner syndrome (45,X) and trisomy X.[17,18] Pallister-Killian syndrome (PKS) is a multisystem developmental disorder caused by tissue limited mosaic tetrasomy of chromosome 12p (isochromosome 12p) and is associated with CDH in 5%–29% of prenatal cases.[19,20]

CNVs

CNVs that range in size from 50 bp to large segmental deletions or duplications of several megabases have been implicated in human disorders that include CDH.[9,14,21] Frequently reported CNVs in CDH include 8p23.1 deletion, 15q26 deletion, 1q41-42 deletion, 4p16 deletion, unbalanced translocation of der(22)t(11:22)(q23:q11).[18] CNVs that encompass multiple genes can have many associated features because of both the pleiotropic effects of single genes on multiple phenotypes and/or the additive impact of multiple individual genes on phenotypes. Analysis of these CNVs have identified candidate genes for CDH (Fig. 1 and Supplementary Table 1). Defining the critical regions by comparing CNVs across CDH patients can assist in identifying relevant CDH genes. Several of the CNVs are not consistently associated with CDH and suggest that the CDH aspect may depend on additional interacting genetic or non-genetic factors.

Deletion CNVs

Deletion of 15q26 is the most frequent CNV associated with CDH, observed in 1.5% of CDH cases.[22,23] Patients with a 15q26 deletion have intra-uterine growth restriction, cardiovascular malformations, CDH and characteristic facial features associated with Fryns syndrome.[22–24] The critical region is 1.7 Mb spanning chr15:96,420,020–98,119,250 (hg19)[25,26] and contains the protein-coding gene: *NR2F2* (nuclear receptor subfamily 2 group F member 2, also known as *COUP-TFII*). *NR2F2* is a transcription factor in the steroid/thyroid hormone receptor superfamily and conditional *Nr2f2* knockout mice develop CDH.[27] NR2F2 interacts with ZFPMs,[28] and is regulated by the retinoid signaling pathway.[29] Pathogenic sequence variants in *NR2F2* have been described in humans with isolated CDH or patients with an atrial septal defect.[30] These genetic data suggest that NR2F2 is a critical gene in the development of CDH in patients with 15q26 deletions.

Deletions of 8p23.1 of variable size are common in CDH (OMIM 222,400).[31] and are frequently associated with cardiac anomalies, neurodevelopmental disabilities (NDD), and facial dysmorphisms.[32,33] The CDH-critical region at 8p23.1 is a 3.7 Mb region between 8,079,861 and 11,860,569 (hg19).[31] *GATA4* and *SOX7* in this critical interval have been implicated in CDH in mice.[34–36] Chromosome 8q22-q23 is recurrently deleted and translocated in individuals with CDH[15,37,38] and is associated with dysmorphic features.[15,38] The minimum deletion is ~1 Mb and includes *ZFPM2*[37] which has been associated with diaphragmatic defects in mice.[39] Inherited or *de novo* damaging *ZFPM2* sequence variants are observed in up to 5% of CDH in a cohort of 275 patients, but the penetrance is reduced in families with *ZFPM2* variants, and the predominant phenotype is usually isolated.[40]

Deletions of 1q41-42 are consistently associated with seizures, NDD, dysmorphic features, and less commonly with a range of birth defects including cleft palate, clubfeet, and 18% with CDH.[41,42] Some patients have a clinical diagnosis of Fryns syndrome including CDH, pulmonary hypoplasia, cleft palate, hypoplastic nails, contractures of the digits, clubfeet, and dysmorphic features.[41,43] The critical region for CDH is 219,914,853–224,637,114 (hg19).[41,43,44] Genes *HLX* (H2.0 like homeobox) and *DISP1* (dispatched RND transporter family member 1) have been implicated in CDH. HLX is a homeobox transcription factor, and the diaphragms of *hlx* null mice were herniated.[45] Heterozygous and homozygous missense variants in *HLX* have been described in CDH patients.[44,46] *DISP1* is highly expressed in the pleuroperitoneal folds (PPF),[47] and a mosaic *de novo* missense variant of *DISP1* in a CDH patient with heart abnormalities and bilateral cleft palate has been identified although the role of Disp1 during diaphragm development is unknown.[47]

The 4p16 deletion causes Wolf–Hirschhorn syndrome (WHS), characterized by typical facial features, skeletal anomalies, intellectual disability, and growth problems. WHS is rarely associated with CDH.[48,49] A 2.3 Mb critical region (chr4:1-2,336,628)[50] contains *FGFRL1* (fibroblast growth factor receptor like 1) and may contribute to CDH.[50,51]

CDH has been reported occasionally in 22q11.2 deletion syndrome (22q11.2DS).[52–54] The 22q11.2 deletion involves

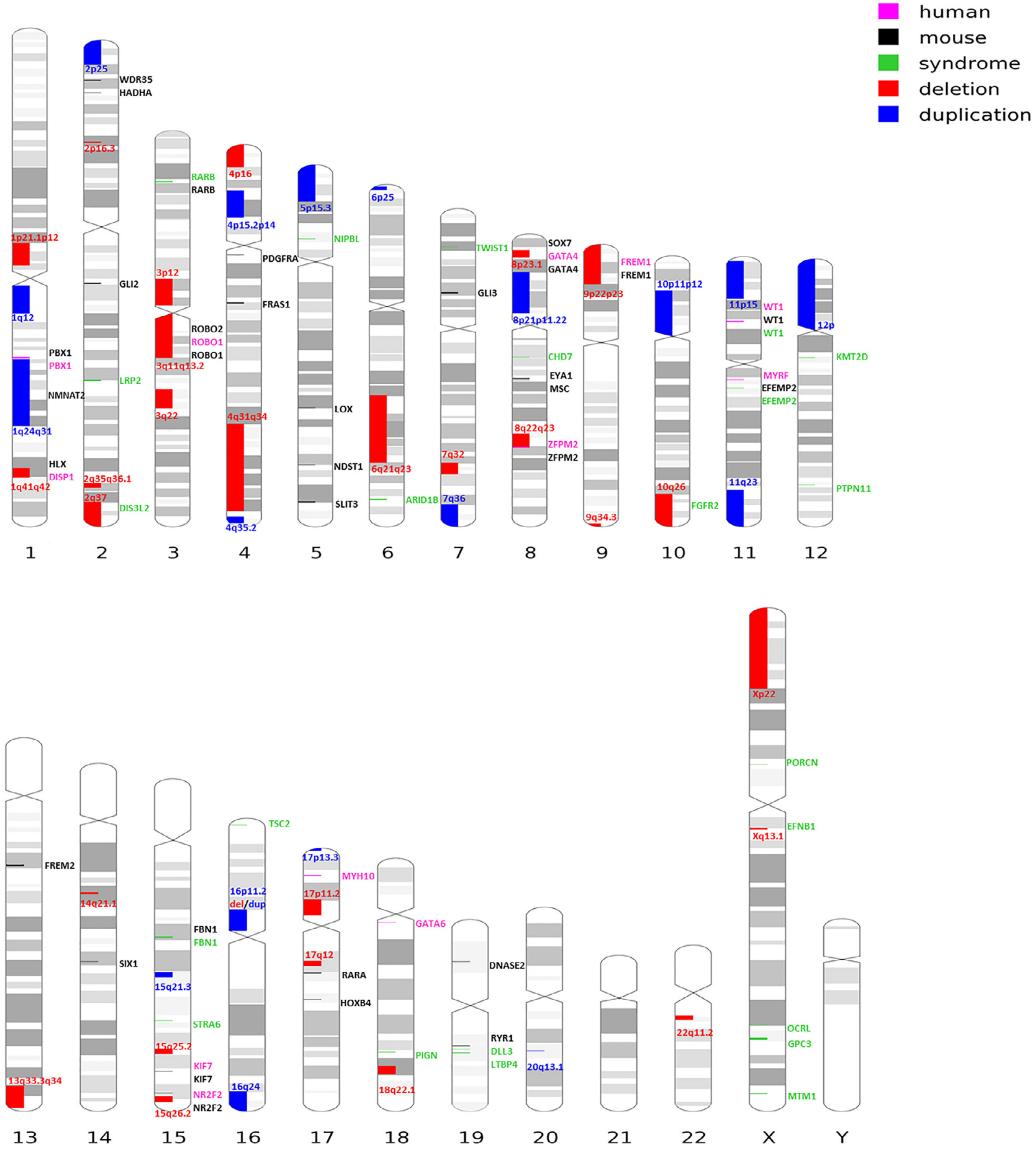

Fig. 1 – Chromosomal anomalies and genes associated with CDH from mouse models and humans. Duplicated (blue) and deleted (red) copy number variants identified in patients with CDH Genes implicated by mouse models (black), monogenetic syndromes (green) and/or patients with isolated CDH or with multiple malformations (purple). Aneuploidies are not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

microdeletions of varying sizes (0.7–3 Mb).[55] A retrospective study of a cohort of patients with 22q11.2 DS revealed that the prevalence of CDH is 0.8%.[17] The hotspot for CDH in chromosome 22 was estimated between chr22:18,640,000–20,680,000 (hg19).[17]

Microdeletion of 17q12 is associated with maturity-onset of diabetes of the young type 5, macrocephaly, developmental delay and renal disease.[56,57] There are six patients with CDH with 17q12 deletion and the minimal region for CDH is a 1.2 Mb deletion from 34,984,100 to 36,207,688 (hg19).[9,56,58,59]

Duplication CNVs

The most frequently implicated duplication in CDH is the 11q23 duplication[60] which usually results from the unbalanced translocations 11;22(q23;q11),[9,61] 11;12(q23;q24),[61] or

11;13(q23;q12.3).[37,62] Ten cases with CDH have been reported to be associated duplications of chromosome 1q.[63] Other duplications include duplications of 8p21-p23.1, 4q31, and 14q32.[15]

Monogenic syndromes associated with CDH

CDH can be isolated but more commonly is an inconsistent feature of a syndrome with other developmental anomalies or neurodevelopmental disabilities. There are more than 70 well characterized syndromes associated with CDH[18,64,65] and over 20 of these syndromes have established genetic causes.[18,65] It is estimated that 3−10% of CDH is associated with an underlying syndromic diagnosis[8,10] which has important implications for prognosis and management. The syndromes can be autosomal recessive, dominant or X-linked.[64] Genes associated with these syndromes are summarized in Fig. 1 and in Supplementary Table 2.

X-linked syndromes

CDH has been reported in 18% of individuals with Simpson-Golabi-Behmel Syndrome [SGBS; OMIM 312,870][66] which is a X−linked recessive disorder characterized by prenatal and postnatal macrosomia, hypertelorism, polydactyly and syndactyly, congenital heart disease (CHD), renal anomalies, intellectual disability, and a predisposition to the development of embryonal tumors.[66] SGBS is caused by microdeletions or missense mutations in *GPC3* (glypican-3).[67] CDH has also been associated with other X-linked recessive disorders, including myotubular myopathy 1 [OMIM: 310,400], which is due to loss of function mutations in the *MTM1* (myotubularin 1) at Xq28[68]; Opitz GBBB syndrome [OMIM:300,000] caused by mutations of gene *MID1*[69] and Lowe syndrome [oculocerebrorenal syndrome; OMIM:309,000] caused by mutations in OCRL (OCRL inositol polyphosphate-5-phosphatase).[70]

Craniofrontonasal syndrome [CFNS; OMIM 304,110] is an X-linked dominant condition characterized by coronal synostosis with hypertelorism, facial asymmetry, a broad and bifid nasal tip and skeletal anomalies. Family studies have shown that the phenotype in males with CFNS is milder than for females with CFNS.[71−73] CDH has been described in both males and females with CFNS.[73] Mutations in *EFNB* 1 (Ephrin B1) cause CFNS.[72] CDH also has been reported in focal dermal hypoplasia/Goltz Syndrome [305,600],[74,75] a X-linked dominant condition with linear pigmentation of the skin, fat herniation, microphthalmia and iris defects, hypoplastic teeth, digital anomalies and bone striation. The syndrome is caused by heterozygous *PORCN* (Porcupine O-Acyltransferase) mutations.[76]

Autosomal dominant syndromes

Cornelia De Lange Syndrome 1 [CDLS1; OMIM 122,470] is a rare and clinically variable disorder affecting multiple organs. It is characterized by distinctive facial dysmorphisms, intellectual disability, and prenatal and postnatal growth restriction. Congenital anomalies include malformations of the upper limbs, gastrointestinal malformation/rotation, pyloric stenosis, CDH, heart defects and genitourinary malformations. A retrospective review of causes of death with CDLS identified CDH as the cause in 10% of patients.[77] Heterozygous mutations in *NIPBL* are the cause of CDLS with CDH.[78,79] Denys−Drash syndrome [DDS; OMIM 194,080] is characterized by urogenital abnormalities, congenital nephropathy, progressive glomerulopathy and the development of Wilms tumors in early childhood. CDH in DDS is rare. Only three cases with *WT1* (Wilms tumor 1) mutations have been described.[80−82] CDH can be seen with other conditions associated with *WT1 mutations*, including Frasier syndrome [FS; MOIM 136,680][83] and Meacham syndrome [OMIM: 608,978].[84] Marfan syndrome [MFS; OMIM:154,700] is a genetic disorder of the connective tissue, with a wide spectrum of clinical variability with skeletal, cardiovascular, pulmonary, and ocular involvement. MFS is caused by mutations of *FBN1* (fibrillin-1) that alter the homeostasis of elastic fibers.[85] Early onset Marfan syndrome (EOMS) is the most severe form of MFS, and approximately 20% of patients with EOMS have a diaphragmatic eventration.[86]

Autosomal recessive syndromes

Donnai−Barrow syndrome [DBS; OMIM: 222,448] is associated with CDH in >50% of patients.[87] It is an autosomal recessive disorder characterized by CDH, exomphalos, absent corpus callosum, myopia, and sensorineural deafness.[88] Biallelic pathogenic variants in *LRP2* cause DBS.[89] LRP2 encodes the multiligand receptor megalin. Megalin ligand vitamin A or retinol[90] is critical for diaphragm development.[91] Microphthalmic syndrome 9 [MCOPS9, 601,186] also known as Matthew-Wood syndrome or Spear Syndrome is another autosomal recessive condition which is caused by biallelic mutations in *STRA6*.[92,93] The encoded protein acts as a receptor for retinol/retinol binding protein complexes.[94] Another retinoic signaling pathway related gene *RARB* (retinoic acid receptor beta) causes syndromic Microphthalmia [MCOPS12; OMIM: 615,524].[95] Both syndromes share similar features of bilateral clinical anophthalmia, pulmonary hypoplasia/aplasia, cardiac malformations, and diaphragmatic hernia/eventration.[95]

CDH genes identified by animal model and humans

Recently, next-generation sequencing technology including whole exome sequencing (WES) and whole genome sequencing (WGS) facilitated the identification of novel single nucleotide variants and genes in sporadic and familial CDH patients.[12,96−98] The genes identified so far are mainly in two broad groups: transcription factors, and molecules involved in cellular migration, or the components of extracellular matrix (ECM)[99] (Table 1). Genetically manipulated mouse models of many of these genes are associated with diaphragm anomalies, further supporting their role in CDH (Supplementary Table 3, Fig. 1).

Transcription factors

Transcription factors involved in retinoic acid (RA) signaling and sonic hedgehog (Shh) signaling pathways implicated in CDH include *WT1, ZFPM2, GATA4, SOX7, GATA6, NR2F2,*

Table 1 – Transcription factors, cell migration and extracellular matrix genes associated with congenital diaphragmatic hernia.

Gene	Gene name	Function	Chromosome location	Genetic variant type in humans	CDH in mice model reference
GATA4	GATA binding protein 4	transcription factor	8p23.1	heterozygous missense[97,108]	[36]
WT1	Wilms Tumor 1	transcription factor	11p13	heterozygous missense[80]	[100,101,138]
ZFPM2	Zinc Finger Protein, FOG Family Member 2	transcription factor	8q23	heterozygous missense/ LGD[40,102,108,139]	[39]
SOX7	SRY-Box 7	transcription factor	8p23.1	N/A	[109]
GATA6	GATA Binding Protein 6	transcription factor	18q11.2	heterozygous missense/ LGD[96,140,141]	N/A
MYRF	Myelin Regulatory Factor	transcription factor	11q12.2	heterozygous missense/ LGD[12,116]	N/A
NR2F2	Nuclear Receptor Subfamily 2 Group F Member 2	transcription factor	15q26.2	heterozygous LGD[108]	[27]
KIF7	Kinesin Family Member 7	transcription factor	15q26.1	heterozygous missense[98]	[142]
GLI3	GLI Family Zinc Finger 3	transcription factor	7p14.1	N/A	[119]
PBX1	PBX Homeobox 1	transcription factor	1q23.3	heterozygous missense[108,122]	[113]
SLIT3	Slit Guidance Ligand 3	cell migration	5q34-q35.1	N/A	[123,125]
ROBO1	Roundabout Guidance Receptor 1	cell migration	3p12.3	heterozygous LGD[143]	[128]
GPC3	Glypican 3	cell migration	Xq26.2	heterozygous LGD[144]	N/A
NDST1	N-Deacetylase And N-Sulfotransferase 1	cell migration	5q33.1	N/A	[130]
FREM1	FRAS1 Related Extracellular Matrix 1	extracellular matrix	9p22.3	biallelic[133]	[133]
FRAS1	Fraser Extracellular Matrix Complex Subunit 1	extracellular matrix	4q21.21	N/A	[134]
FREM2	FRAS1 Related Extracellular Matrix Protein 2	extracellular matrix	13q13.3	N/A	[134]

CDH, congenital diaphragmatic hernia; LGD, likely gene disrupting, N/A, not available.

MYRF, KIF7, GLI3, and *PBX1*. *WT1* is involved in RA signaling pathway and is expressed in PPF.[7] Heterozygous mutations in *WT1,* cause a series of overlapping clinical syndromes that can include CDH. *Wt1* null mice exhibit diaphragmatic hernia.[100,101]

ZFPM2 (also called *FOG-2*, friend of GATA-2) is located in a CNV recurrently associated with CDH. Fog2$^{-/-}$ mice generated through chemical mutagenesis have been shown to have diaphragmatic eventration and pulmonary hypoplasia.[39] In humans, inherited and *de novo* variants in *ZFPM2* gene were identified predominantly in isolated CDH patients.[39,40,102]

GATA4 and *SOX7* are both located on chromosome 8p23.1. *GATA4* interacts with *ZFPM*[103] and is an important component of the retinoic signaling pathway.[104] *Gata4* mice have cardiac, lung or diaphragm defects.[36] In humans, mutations in *GATA4* have been associated with congenital heart defects[105,106] and CDH.[107,108] Sox7 is another transcription factor. Haploinsufficiency of Sox7 can produce anterior CDH in mice.[109] No mutations in human CDH patients have yet been identified in *SOX7.*

Several lines of evidence suggest that haploinsufficiency of NR2F2 causes human CDH. *NR2F2* is expressed in diaphragm, lungs and heart during embryonic development,[27] and expression is regulated by the retinoid signaling pathway.[110] Homozygous tissue-specific ablation of NR2F2 in mice cause posterolateral CDH.[27] Furthermore, NR2F2 physically interacts with ZFPM2, suggesting these two factors together with GATA4 may cooperate in diaphragm development.[28]

Several *de novo* and inherited damaging variants in *GATA6* have been identified in CDH patients,[111] many with additional cardiac or pancreatic malformations.[112] In mice, GATA6 is expressed in the septum transversum mesenchyme and the lateral PPFs.[113,114] *Gata6* expression was significantly decreased in PPF2 and in muscularized diaphragms in the nitrofen-induced CDH model.[115] Decreased *Gata6* expression results in the reduction of proliferating mesenchymal cells in nitrofen-exposed fetuses, causing malformed PPFs and consequently leading to diaphragmatic defects in the nitrofen-induced CDH model.[115]

De novo variants in Myelin regulatory factor (MYRF) recently have been reported in patients with phenotypes including CDH, CHD and urogenital anomalies,[12,116,117] suggesting a new syndrome caused by *MYRF* mutations. MYRF is a membrane associated transcriptional factor which was originally identified to be essential for the development and maintenance of central nervous system myelination.[118] It is highly expressed in the developing diaphragm.[113] Analyzing the transcriptome of patient-derived diaphragm fibroblast cells suggest that disease associated variants are due to loss of function.[12]

The sonic hedgehog (Shh) signaling pathway has been shown to be involved in diaphragm development.[113] The Shh transcription factors *GLI2* and *GLI3* knockout mice have

CDH.[119] *KIF7 (*kinesin family member 7) and PBX1 (PBX Homeobox 1) are regulators of Gli3, a transcription factor in the Shh signaling pathway.[120] Homozygous Kif7 mice[121] and Pbx1 knockout mice have diaphragmatic defects.[113] Haploinsufficiency of *PBX1* has recently been associated with multiple developmental defects including CDH.[122] Two rare and predicted pathogenic variants in KIF7 gene were identified in human CDH patients.[98]

Cellular migration, or the components of extracellular matrix (ECM)

Genes involved in cell migration, myogenesis or extracellular matrix formation are critical to normal diaphragm development.[113] Mutations in genes such as *SLIT3, ROBO1, GPC3, NDST1,* FREM1, *FRAS1,* and *FREM2* are associated with CDH. *SLIT3* (slit guidance ligand 3) is one of the three human homologs of the Drosophila *Slit* gene. The slit proteins are secreted molecules with multiple roles in cell migration and adhesion through interacting with roundabout (Robo) homolog receptors to affect cell migration.[123,124] *SLIT* genes are expressed in the central nervous system during development, but *SLIT3* is also expressed in the mesothelium of the diaphragm during embryonic development.[123] The majority of *Slit3* deficient mice developed central-type diaphragmatic hernias.[123,125] No SLIT3 mutations have been reported in humans with CDH to date. *ROBO1* encodes a surface membrane protein that functions in axon guidance and neuronal precursor cell migration and cardiac development.[126,127] Most *Robo1* homozygous mice died at birth of respiratory failure because of delayed lung maturation, and ~6% of homozygous mice also exhibited CDH.[128] Recent studies identified a second Slit receptor heparin sulfate as an integral component of Slit-Robo signaling complex which stabilized the Slit-Robo-interaction.[129] Mutations in a heparin sulfate proteoglycan, GPC3, cause the CDH associated syndrome SGBS (discussed above). Loss of another heparin sulfate biosynthetic enzyme *NDST1* in mice also show defective diaphragm development.[130]

The FRAS/FREM extracellular matrix (ECM) proteins (*FRAS1, FREM1* and *FREM2*) form a complex and mediate cell adhesion and intercellular signaling during embryonic development.[131] The FRAS1 related extracellular matrix1 (*FREM1*) gene encodes an extracellular matrix protein that plays a critical role in the development of multiple organs including diaphragm.[132] Recessive loss-of-function mutations affecting *FREM1* have been shown to cause isolated CDH in humans and up to 47% of *Frem1* homozygous mice have CDH.[133] Biallelic variants were identified in a CDH cohort.[134] Diaphragmatic hernia was seen in 8% of Frem2-deficient mice and 1% of Fras1-deficienct mice.[134] Loss of the FREM1/FREM2/FRAS1 complex is thought to result in anterior sac CDH through its effects on mesothelial fold progression.[134]

Other genes identified by sequencing

Due to the historically high mortality with CDH, few familial studies of CDH have been conducted because of decreased reproductive fitness. The low recurrence rates[135] among siblings and the lack of family history in the majority of CDH patients with CDH support the model of *de novo* variants causing a substantial fraction of CDH, especially syndromic cases with low reproductive fitness. Exome/genome sequencing has supported the identification of new genes for CDH, often due to *de novo* mutations.[11−13] Recent studies identified *de novo* and inherited variants in *GATA4*,[97] *GATA6*,[96] *ZFPM2*,[40] and *MYH10*[136] for CDH (Fig. 1). In the case series to date, few genes had more than two independent deleterious variants, suggesting that there is significant heterogeneity in genetic etiology, similar to congenital heart disease and neurodevelopmental disorders.[12] Prioritizing among identified *de novo* or inherited rare variants is a challenge for the genes that are not recurrent. Studies across diseases may be helpful since many genes identified with deleterious *de novo* variants are highly enriched at the top quartile of expression in developing diaphragm, heart and brain,[11-13] suggesting pleiotropy in complex cases. Since CHD and NDD are the most common syndromic features in CDH, evidence from genes involved across these conditions may help to prioritize risk genes in CDH. For example *STAG2*, which encodes a subunit of the cohesin complex and has been associated with intellectual disability and behavioral problems.[137]

In our prior studies, 21% of complex cases and 12% of isolated CDH cases are attributable to *de novo* likely gene-disrupting or deleterious missense variants.[12] Inherited coding variants, *de novo* noncoding variants, and somatic variants may also contribute to CDH and are currently being assessed[64] with genome sequencing and RNA sequencing.

Genetic counseling

Prenatal ultrasound screening enables early CDH diagnosis. Assessment of the family history should include prenatal or neonatal deaths of uncertain cause, congenital anomalies and NDD in family members. Diaphragm defects in other family members can be subtle, and MRI imaging in parents can identify small hernias or eventrations that are clinically asymptomatic but that suggest a higher risk of recurrence due to incomplete penetrance.[40,96,97] Perinatal genetic data from chromosome microarray and fetal exome/genome sequencing may enable diagnosis of a genetic syndrome and refine prognosis and inform clinical management. But even with a comprehensive genetic evaluation, a definitive genetic cause may not be identified in the majority of CDH cases. In approximately 30% of non-isolated CDH cases a genetic etiology can be identified with a combination of karyotype, chromosome microarray and trio exome sequencing.[8−13] Most of large chromosome anomalies are diagnosable with a conventional karyotype. Chromosome microarray detects small genomic deletions and duplications and offers incremental yield over karyotyping and is recommended in clinical evaluation. Chromosome anomalies are detected in ~10% of patients with CDH with standard clinical karyotype and chromosome microarray.[8] Exome sequencing including both parents is recommended, especially in patients with multiple anomalies or NDD. Approximately 10−22% of CDH patients have a *de novo* sequence variant that is a major contributing factor to CDH.[11−13,98] Because we do not yet know all the genes causing CDH, reanalysis of the sequence data in the future may be helpful even if a diagnosis is not established.

As *de novo* deleterious variant are identified in novel genes not previously associated with human disease, an immediate question is whether the identified genetic variant is expected to be associated with isolated CDH or to have pleiotropic effects on other organs such as the lungs, heart and brain. We use data from mice with comparable mutations and gene expression data across tissues to predict outcome based upon the type of gene implicated and phenotypes associated with mutations in other similar genes; however, we cannot always accurately predict the prognosis.

Identifying an established genetic etiology clarifies the recurrence risk for the family. The recurrence risk for parents to have another affected children after having one child with CDH is about 2% in families without a known family history or known genetic etiology.[122] A *de novo* genetic cause has a 1% recurrence risk for the parents but up to a 50% recurrence risk for the individual carrying the genetic mutation.

Conclusion

With the availability of new molecular genetic techniques to facilitate gene discoveries for CDH, our understanding of the role of genetics in the pathogenesis of CDH may make it possible to use preventative strategies and therapeutic interventions to partially alleviate the pulmonary disease in patients with CDH. Because CDH is so genetically heterogeneous, further studies need to include analysis of larger numbers of CDH patients using exome/genome sequencing, single cell sequencing or RNA sequencing of the developing PPF, bioinformatics analysis, and animal models to identify additional pathogenic genes and variants and determine the associated clinical features and outcomes.

Declaration of Competing Interest

There is no conflict of interest for all authors.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.semperi.2019.07.008.

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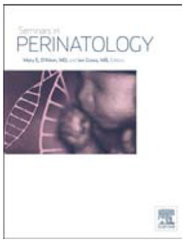

Basic and translational science advances in congenital diaphragmatic hernia

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ABSTRACT

Congenital Diaphragmatic Hernia (CDH) is a birth defect that is characterized by lung hypoplasia, pulmonary hypertension and a diaphragmatic defect that allows herniation of abdominal organs into the thoracic cavity. Although widely unknown to the public, it occurs as frequently as cystic fibrosis (1:2500). There is no monogenetic cause, but different animal models revealed various biological processes and epigenetic factors involved in the pathogenesis. However, the pathobiology of CDH is not sufficiently understood and its mortality still ranges between 30 and 50%. Future collaborative initiatives are required to improve our basic knowledge and advance novel strategies to (prenatally) treat the abnormal lung development. This review focusses on the genetic, epigenetic and protein background and the latest advances in basic and translational aspects of CDH research.

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Introduction

Congenital diaphragmatic hernia (CDH) is a severe and common congenital anomaly of unknown origin that is characterised by pulmonary hypoplasia, pulmonary hypertension and a diaphragmatic defect that can differ in size and location. Although generally unknown to the public, this disease occurs as frequently as cystic fibrosis (incidence = 1:2500 newborns), hence every 10 min a baby is born with CDH worldwide. Although neonatal intensive care and neonatal surgery have improved outcomes for CDH babies significantly, 30–50% of these babies still die mainly due to the underlying pulmonary hypoplasia and hypertension.[1] The lung hypoplasia is characterized by thickened mesenchyme, decreased airway branching and less alveolarization, whereas pulmonary hypertension results mainly from hypermuscularized pulmonary arteries and vascular remodelling. The combination of both leads to impaired gas exchange, hypoxia and unfortunately lethal cardiac or respiratory failure in the most severe cases.

The diagnosis can be made prenatally with 70% sensitivity via ultrasound and fetal MRI,[2] but prenatal outcome prediction, which is currently completely based on imaging modalities, remains imperfect. A prenatal treatment to improve lung maturation is not established but randomized controlled trials for fetoscopic endoluminal tracheal occlusion (FETO) therapy are currently underway. In this procedure, the fetal

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trachea is plugged with a balloon for approximately 4 weeks and the consequent accumulation of lung fluid and increased pressure in the airways can promote pulmonary growth.[3]

In contrast to cystic fibrosis, where the discovery of the CFTR mutation and significant research funding helped to uncover many of the underlying pathways, the pathogenesis of CDH remains unclear. A monogenetic origin has not been identified, suggesting that epigenetic and environmental factors may be important in the pathogenesis. Only up to 30% of cases can be explained by a genetic cause[4] and discordance between monozygotic twins and recurrence rates of 0.7% among siblings support the theory that CDH is rather multifactorial than monogenetic in nature.[5] Cases of isolated CDH in otherwise healthy infants warrant the search for better opportunities to improve prenatal lung development. This would decrease the burden of affected families, but also health care systems, since long and expensive intensive care treatment and long-term cardiopulmonary, neurodevelopmental, and nutritional sequelae come with a very high cost per patient. The annual cost that is associated with complications due to CDH can range from $160.000 to $810,000 in the United States.[6] In Canada, the economic burden due to CDH is up to four times greater than other illness-severity and age-matched neonates.[7]

Different animal models have been used to study CDH, including larger models with surgically induced diaphragmatic defects (sheep, rabbit), transgenic mouse models or pharmacological models.[8] The commonly accepted nitrofen rat model can mimic the human disease to a large degree, whereby the application of the herbicide nitrofen to pregnant rats causes pulmonary hypoplasia (100% occurrence), pulmonary hypertension and a Bochdalek type diaphragmatic defect (60%–70% occurrence) in the fetuses.[9–11] This provides evidence that environmental factors can cause CDH in vivo, although nitrofen has not been associated with human CDH. Embryonic studies with this model led to the *dual-hit hypothesis*, based on observations of a primary insult to both lungs before the diaphragmatic closure occurs and a second hit to the ipsilateral lung that results from herniation of abdominal organs into the thoracic cavity.[12] That insight shifted the "classical paradigm" from a theory of mechanical compression of the lungs and subsequent interference with fetal breathing movements and lung growth towards a more complex view, where impaired lung development occurs somewhat independently from the diaphragmatic defect. This raises many questions about a dysregulation of the developmental pathways that are involved in both lung and diaphragmatic development, and if there is an environmental factor that can cross the placenta and cause damage to both organs. More recent advances in "omics" technologies has allowed for a better understanding of the underlying pathways and provided a broader view on genome, epigenome and proteome level changes (as highlighted below), but the overall pathogenesis of CDH remains unclear.

Previous excellent reviews have focussed on the genetic basis of CDH[13–15] (see article in this issue of the *Seminars*), the different animal models,[8] the proteins associated with pulmonary hypoplasia or hypertension in the nitrofen rat model,[9,16] or have provided an overview of the developmental changes of diaphragm and lungs.[17] Herein, we give an overview of the recent advances in basic science on CDH and highlight the translational aspects from bench to bedside (also see Fig. 1). We tried to provide a new perspective by focusing on insights at different levels of cellular biology according to the paradigm of DNA level, RNA level and protein level.

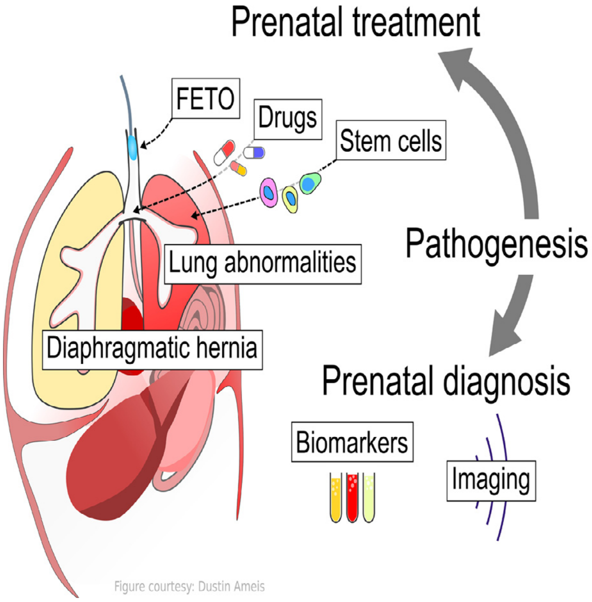

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Fig. 1 – Schematic representation of the different aspects of basic science and translational CDH research.

Advances in genetic CDH research

Most CDH cases are isolated in nature, and do not occur in association with other anomalies.[13] Congenital heart defects are the most commonly associated malformations (15–20% of the cases).[18,19] Although 70% of CDH cases cannot be attributed to genetic aberrations, the remaining 30% are related to different, known genetic causes, including single gene mutations, copy number variants or aneuploidies.[15] The genetic causes of CDH have been extensively reviewed elsewhere[13–15] (and within this issue of *Seminars*) and therefore only some of the more recent genetic associations in CDH are highlighted here. Examples of syndromes associated with CDH are: Cornelia de Lange, Beckwith-Wiedemann, Fryns, Denys Drash, and many others.[20]

Most recently, Qi et al. reported a novel *de novo* variant in *MYRF* among CDH patients.[21] The authors performed whole exome or genome sequencing on 362 patient-father-mother triads and observed damaging alterations of *MYRF* in 4 patients. Next, they found common syndromic phenotypes in 12 patients with MYRF *de novo* variants suggesting that this is a novel gene locus associated with CDH and other abnormalities including heart defects and genitourinary malformations. Since penetrance for CDH was incomplete compared to a complete penetrance for heart defects, the authors concluded that other epigenetic or environmental factors are important for CDH development. The *MYRF* gene adds to the known CDH genes *ZFPM2, FOG2, GATA4*[22] and *GATA6*.[23]

CDH-associated genes show incomplete penetrance and therefore cause different diaphragmatic defects.[13] Using whole exome sequencing, Chung and colleagues showed that CDH can be associated to an inherited missense mutation of *GATA4*. Screening of patients revealed also de novo *GATA4* variants in a syndromic CDH patient.[22] Interestingly, disruption of *GATA4* was also observed in the nitrofen rat model, where expression of *GATA4* is decreased in the developing diaphragm of CDH offspring.[24] Moreover, Yu et al. showed that *de novo* mutations in *GATA6* can be associated with CDH, pancreatic agenesis, and congenital heart defects.[25] Likewise, this could be confirmed in the nitrofen rat model, where *GATA6* expression was significantly decreased in the developing lung and diaphragm after nitrofen exposure.[26] This exemplifies the relevance of the nitrofen rat model and suggests similarities between human and nitrofen-induced rodent CDH.

Advances in epigenetic CDH research

Epigenetics is defined as the study of heritable phenotype changes that occur without structural alterations to the DNA sequence. While the DNA sequence defines our genome, RNA molecules known as gene transcripts can translate our genetic information into proteins. This translational process can be influenced by epigenetic regulators such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs) or circular RNAs (circRNAs). Moreover, DNA methylation or histone modifications are important epigenetic factors that regulate gene expression, but surprisingly these processes have not been explored adequately in CDH research.

Several groups have highlighted the importance of epigenetic alterations in CDH. Russel et al. performed whole transcriptomic analysis of the developing mouse diaphragm to identify novel candidate gene transcripts that are likely to be involved in the pathobiology of CDH.[27] Using bioinformatic analysis, they compared their targets to previously CDH associated genes from human and murine data. This revealed a novel candidate gene, pre-B-cell leukemia transcription factor 1 (*Pbx1*) and subsequent knock out of *Pbx1* led to diaphragmatic defects in a mouse model. The authors argue that such “prioritization strategies” together with knowledge from previous human and animal data, can predict additional disease-associated genes. Burgos et al. studied the transcriptome of nitrofen-induced hypoplastic lungs and found that several mRNA transcripts were dysregulated in CDH.[28] The most important differentially expressed transcripts included the FGF family, TGF superfamily, other transcription factors and angiogenesis factors. Mahood et al. used RNA sequencing from nitrofen exposed hypoplastic lungs (E13.5) and found that the most enriched pathways within hypoplastic lungs were Wnt signalling, Cadherin signalling, and inflammatory processes.[29] Engels et al. used a surgically induced rabbit model for CDH and compared the transcriptomes between control fetuses, fetuses with CDH and fetuses with CDH treated with tracheal occlusion. They found a significant transcriptional upregulation of the ciliated cell marker Forkhead box protein J1 (*FOXJ1*) in CDH rabbits, whereas TO was able to reverse *FOXJ1* upregulation.[30] Using an *ex vivo* compression model of CDH, Fox et al. found that alpha smooth muscle actin (*ACTA2*) gene expression was increased when lungs were treated with nitrofen and externally compressed with a device.[31] Conversely, they found that transcripts of periostin and surfactant protein C were significantly decreased by external compression and nitrofen treatment.

More recently, different micro RNAs (miRNA or miR) have been studied in CDH. Micro RNAs are small RNA molecules of 21 - 25 nucleotides, that can bind messenger RNAs and thereby silence gene expression.[32] With this function they have significant influence on cellular transcription and regulate developmental and physiological processes. We reported that miR-200b is significantly dysregulated in human and experimental CDH. Survivors born with a severe type of CDH show prominently increased levels of miR-200b in their tracheal fluids after FETO compared to non-survivors, indicating a protective effect of miR-200b in these individuals.[33] Similarly, miR-200b is upregulated in CDH lungs (alveolar stage) and necessary for normal distal airway development.[34] Moreover, prenatal transplacental miR-200b treatment of pregnant nitrofen rats decreased the incidence of CDH by approximately 30% and improved fetal lung morphology and alveolarization.[35] Dysregulation of miR-200b was also observed in the rabbit model of CDH, where hypoplastic lungs demonstrated upregulation of miR-200b, confirming its importance in different species.[36] We interpret these results as a compensatory upregulation of miR-200b that is important for lung maturation with beneficial effects on pulmonary hypoplasia.

Additionally to our observations on miR-200b, Zhu et al. studied differential expression of miRNAs in the nitrofen model at late stages of lung development and found a significant decrease in miR-33 in nitrofen-exposed lungs.[37] Herrera-Rivero et al. showed, that circulating miRNAs can be associated with the development of chronic lung disease and pulmonary hypertension in newborn CDH babies.[38] In patients with less favorable outcomes, 7 miRs were significantly altered compared to less severe cases and target gene pathway analysis revealed the regulation of cytokines, growth factors, and cellular stressors by these miRNAs. Their study suggests, that miRNA profiles can be used to discover biomarkers for pulmonary hypertension severity and better determine individual outcomes of CDH babies.

Other RNA molecules, like circRNAs or lncRNAs have not been reported in the context of CDH. We recently profiled circRNAs in human and nitrofen rat lungs and found significant alterations, suggesting that other levels of epigenetic regulators are worth exploring (unpublished data). Furthermore, we are currently exploring the potential of circRNAs as predictive outcome parameters for CDH survival. Overall, the focus on epigenetic changes in developmental diseases has become more prominent over the last decade. Unbiased omics approaches to profile certain types of molecules in diseased organs and comparison to controls has a great potential to increase our understanding of the pathobiology of CDH.

Advances in protein CDH research

Despite many advances of knowledge about the genomic alterations in both experimental and human CDH, the investigation of changes occurring at the protein level is essential for a better understanding of the mechanisms leading to pulmonary hypoplasia. Proteins are the main effectors of pulmonary physiology by regulating signaling between cells, defining lung architecture, and allowing efficient pulmonary function.

Fetal lung development originates from permanent and coordinated interactions between pulmonary epithelial and mesenchymal layers through several signaling pathways.[39] In the nitrofen model of CDH, most of the signaling pathways crucial for fetal lung development have been reported as decreased at the protein level compared to controls. These downregulated pathways include fibroblast growth factors, bone morphogenetic pathway, sonic hedgehog, Wnt pathway, insulin growth factor, connective tissue growth factor, and others.[9] Signaling proteins secreted from epithelial and mesenchymal cells are decreased, resulting in overall reduced epithelial-mesenchymal interactions and a delay in lung growth and maturation (pulmonary hypoplasia). Similarly, many studies have reported the disruption of several signaling pathways leading to vascular remodeling in the nitrofen-induced CDH model.[16]

Since the discovery in 1949 that maternal vitamin A deficiency results in CDH in fetal rats, the vitamin A (retinoic acid) signaling pathway has been studied in depth in several experimental models and plays a key-role in the pathogenesis of CDH and pulmonary hypoplasia.[40] In fact, the overexpression of one of the main enzymes responsible for conversion of retinol to the active component retinoic acid, ALDH1A2, in lungs of both rabbits with surgically-induced CDH and human fetuses is thought to be secondary to lung injury caused by CDH.[41]

Transforming Growth Factor-β is the only growth factor that has been studied in surgically-induced CDH models and the downstream signaling pathway is unaffected in lungs of rabbit fetuses with CDH.[42,43]

The pulmonary extracellular matrix (ECM), that includes more than 150 proteins and enzymes, determines lung architecture and provides mechanical stability and elasticity. Initially considered as an inert layer of the lung, resulting from the accumulation of proteins secreted by epithelial and mesenchymal cells, it is now widely accepted that the ECM is a highly dynamic structure, essential to cellular interactions and playing a key role in lung development.[44]

Collagen is the main ECM component responsible for the tensile strength of the lung and is increased in both the nitrofen and surgical model models of CDH.[45,46] This increased abundance of collagen within the pulmonary ECM structure can possibly lead to thickened and stiffer lungs. Conversely, elastin provides the elastic recoil of the lung and has been reported to have a disorganized distribution in lungs of several models of CDH as well as lungs of human fetuses with CDH, with less elastin at the tip of secondary septation crests, but an accumulation of elastic fibers within the thickened alveolar walls.[47–49]

Several other ECM components, such as integrins, tenascin, fibulin and lysyl oxidase have been reported as having lower levels in experimental CDH than control lungs.[48] Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are enzymes involved in the thinning of the pulmonary mesenchyme and the formation of functional alveoli. Although the role of the MMP/TIMP system is still unclear in the pathogenesis of pulmonary hypoplasia secondary to CDH, an increased MMP/TIMP ratio has been reported in the nitrofen model and lungs of human fetuses with CDH, potentially leading to disrupted ECM and remodeling of the interstitium and the pulmonary vasculature.[50–52] Whereas most of MMPs are expressed mainly in alveolar epithelial cells in physiological conditions, these proteins were also expressed in mesenchymal cells of the thickened alveolar mesenchyme in rabbit fetuses with CDH.[48]

Pulmonary surfactant is essential to prevent alveolar collapse during breathing by reducing air-liquid surface tension. Surfactant secretion by alveolar type 2 (AT2) cells requires the assemblage of 2 components: proteins, produced in AT2 cells; and lipids, transferred from lipofibroblasts to AT2s. Although surfactant deficiency is the main cause of respiratory distress in preterm neonates, it is still controversial whether surfactant deficiency is present in lungs of CDH neonates and participates in pulmonary dysfunction of these infants.

In the early years of experimental CDH research, deficiency in surfactant proteins (SP) has been repeatedly reported in surgical models of CDH.[53–55] However, more recently, SP-B has been reported as either unchanged or increased in both rabbit and lamb CDH lungs compared to controls, possibly due to different techniques for quantifying the presence of these proteins.[46,56–58] In the nitrofen model of CDH, although there is initially a delay in production of surfactant protein B and C in the pseudoglandular stage of lung development, conflicting results have been reported regarding the saccular stage in this model.[59] Whereas some studies reported a decreased expression of SPs in whole lungs,[60–62] studies that focused on distal airways reported an increase in SPs, suggesting a sufficient production of surfactant protein in the distal airways, where surfactant is assembled from its lipid and protein component.[63,64]

However, several factors involved in the synthesis and maturation of the lipid component of surfactant are decreased in nitrofen-exposed lungs, associated to an impaired differentiation of lipofibroblasts, responsible for lipid synthesis, stockage and transfer to AT2 cells.[65–67] Similarly, a decrease in phoshatidylcholine in bronchoalveolar lavages of sheep with CDH has been reported.[68] Despite this deficit in the main lipid component of surfactant, prophylactic treatment of sheep fetuses with surfactant did not improve ventilation of these lambs,[69] suggesting that surfactant deficiency does not seem to be a participating factor in the development of respiratory distress of these lambs.

Finally, the content and maturation of pulmonary surfactant has been reported as similar in lungs of human fetuses with or without CDH.[70] A multicenter study from the CDH Study Group showed surfactant administration to term neonates with CDH had no beneficial effect on the outcome of these infants,[71] suggesting that the observations made in experimental CDH regarding the absence of surfactant deficiency are similar in human CDH.

Translational advances in CDH research

Basic science is essential to advance the prevention, diagnosis, and treatment of diseases and ultimately to improve patient care. Although scientific discoveries have improved our understanding of human (patho-) biology fast, translation into clinical practice settings is often unsatisfactory. The so-called “valley of death” between biomedical research and clinical applications in patient care has widened considerably over the last decades.[72] As an example, the translation rate for cancer research from animal models to clinical trials approaches 8%.[73] In CDH basic science investigation, as in other research areas of pediatric surgery, most progress has been made in novel surgical approaches for the hernia repair (thoracoscopy vs. open surgery) or clinical research addressing clinical strategies such as respiratory strategies in postnatal intensive care.[74] As an example, Harrison and coauthors showed in the early 1980s that fetal surgical hernia repair was feasible in animals. He then tried to translate this approach to treat the human CDH fetus prenatally, but later focused on fetal tracheal occlusion as a potential alternative to open fetal surgery.[75,76] These achievements led to the introduction of fetoscopic tracheal occlusion that can be performed using minimally invasive techniques. Unfortunately, the translation of basic scientific findings from the laboratory to the clinic has been rather slow. Since surgical hernia repair is relatively safe and mortality depends almost entirely on the underlying pulmonary hypoplasia and hypertension, the main goal of basic science is to improve lung maturation, ideally before birth.

Current prenatal surgical interventions to promote lung growth are based on the observation that babies with prenatal high airway obstruction develop larger lungs, which led to the hypothesis that occlusion of the fetal trachea may promote pulmonary development in CDH. In 1994, DiFiore et al. showed that prenatal tracheal ligation in a sheep model resulted in improved lung development.[77] At first, a clip was used to obstruct the fetal trachea, but this procedure was replaced in 2004 by percutaneous fetoscopic endoluminal tracheal occlusion (FETO).[78] During this procedure, a balloon is placed and inflated in the fetal trachea, resulting in pulmonary fluid accumulation and subsequent lung growth. A second percutaneous intervention is required 4–6 weeks later to remove the balloon from the trachea before birth. This procedure is currently being evaluated by clinical trials in Europe, the US, Canada, and Australia (https://totaltrial.eu), which exemplifies the translation from bench to bedside. A systematic review, including 5 studies on prenatal FETO showed promising results with an increased survival in severe isolated CDH cases.[3] More recently, a replacement of the balloon by a self-dissolving fibrin gel plug in a rabbit model has been proposed as another advancement of this technique, thus avoiding a second intervention for balloon removal.[79]

Whereas FETO can primarily correct pulmonary hypoplasia, another hallmark of translational CDH research is to find an antenatal therapy to improve pulmonary hypertension (PH). Vascular remodelling, endothelial-to-mesenchymal transition, and inflammation contribute largely to PH in children and adults.[80,81] In the pediatric population it can occur due to different underlying conditions, e.g. bronchopulmonary dysplasia, alveolar capillary dysplasia or CDH.[82] An ideal prenatal medical treatment for pulmonary hypertension would have positive effects to the fetal lung via administration to the mother and have no side effects for the fetus or the mother. Sildenafil with its known vasodilatory effects via inhibition of phosphodiesterase 5 (PDE-5) has therefore been tested to counteract vascular remodelling and improve angiogenesis in CDH.[83] Luong et al. first showed that sildenafil could improve the pulmonary vasculature and decrease right ventricular hypertrophy in the nitrofen model.[84] Importantly, sildenafil did not interfere with the development of other PDE-5-expressing organs suggesting its effectiveness and potential safety as a targeted approach. Single-dose intra-amniotic sildenafil treatment also normalized the vascular smooth muscle cell phenotype in a mouse model of CDH.[85] Others confirmed the positive effects of prenatal sildenafil on the pulmonary vascular development and showed that additional simvastatin can restore cardiac myocyte appearance and cardiac morphology.[86] Deprest and colleagues confirmed the positive effects of sildenafil in a rabbit model for CDH, but they also reported detrimental effects on vascular branching in normal fetal rabbit lungs.[87] Despite other promising results in preclinical studies with sildenafil,[88] the recent suspension of the STRIDER trial where 11 babies died due to pulmonary abnormalities after prenatal sildenafil treatment for intrauterine growth retardation was a horrifying drawback for this approach and prenatal sildenafil treatment for CDH has been put on hold since then.[89] This demonstrates the importance of sufficient preclinical testing of drug use, especially in the field of perinatal medicine. Future research will have to show whether the initial promising preclinical prenatal treatment studies with sildenafil can be safely translated to clinical trials in human CDH cases.

Other approaches to improve lung development include novel therapeutic targets such as microRNAs or different drugs that were previously tested for other diseases. Using the nitrofen rat model, we showed that prenatal miR-200b treatment improved lung hypoplasia and decreased CDH occurrence significantly.[35] Dysregulation of mir-200b has been observed in human CDH and the nitrofen rat model, but the translation of prenatal miR-200b treatment in human CDH can only be evaluated when negative side effects or potential off target effects of miR-200b to other organ systems have been ruled out. Gosemann et al. recently reported that prenatal rosiglitazone administration, a monocyte chemoattractant protein −1 (MCP-1) antagonist, can mitigate vascular remodelling in the nitrofen model and decrease monocyte influx into the arterial walls.[90] Others showed that prenatal inhibition of macrophage migration inhibitory factor (MIF) by ISO-92 can improve pulmonary development and angiogenesis in nitrofen-exposed rats.[91] These authors showed that right ventricular systolic pressure and vascular wall thickness of pulmonary arteries is restored to normal after nitrofen treatment together with ISO-92. Inhibition of MIF activity was also associated with increased abundance of vascular endothelial growth factor (VEGF), a protein crucially involved in pulmonary angiogenesis. Santos et al. reported that the growth hormone ghrelin is overexpressed in hypoplastic CDH lungs.[92] The antenatal exogenous application of ghrelin reduced lung hypoplasia without effects on the growth hormone secretagogue receptor.[93] Eastwood et al. studied Glucagon-Like Peptide-1 for the same purposes in the CDH rabbit model and found improved peripheral pulmonary vasculature development, but also reported significant maternal side effects opposing its use as a medical strategy.[94] Prenatal administration of vitamin A and retinoic acid to pregnant rats decreased CDH incidence from 54% to 32%,[95] which supports several reports of disruption of the retinoid system in CDH. However, retinoic acid is a known teratogen agent, and prenatal administration may result in severe fetal defects, including central nervous system malformations, preventing its use as a prenatal treatment for pulmonary hypoplasia.[96]

Finally, in the search for novel strategies for both lung regeneration and diaphragmatic repair, stem cells and their derivatives have recently shown promising results.[97,98] Whereas clinical trials involving the use of stem cells have already begun for bronchopulmonary dysplasia - a neonatal lung condition affecting preterm babies - the use of stem-cell derived therapies in pulmonary hypoplasia is still experimental.[99] The amniotic fluid is an ideal source of stem cells for fetal and neonatal disorders as fetuses diagnosed prenatally with a structural defect, such as CDH, will routinely undergo amniocentesis for karyotyping.[100] As such, stem cells derived from the amniotic fluid have been used to promote lung regeneration in experimental CDH. Amniotic fluid stem cells (AFSCs) are multipotent stem cells that are able to differentiate into various cell lineages belonging to the three germ layers. AFSCs were first isolated from amniotic fluid in 2007 and are characterized by the expression of the surface antigen c-Kit.[101] Pederiva et al. were the first to demonstrate the ability of AFSCs to regenerate nitrofen-exposed hypoplastic lungs of CDH rat fetuses.[102] Based on the low cellular engraftment rate into the lung tissue, the authors suggested that their beneficial effects occurred primarily through a paracrine mechanism.[102] This was confirmed by Di Bernardo et al., who proved that the similar effects of lung regeneration could be obtained with the conditioned medium from amniotic fluid-derived mesenchymal stromal cells (MSCs).[103] Interestingly, the authors did not notice any beneficial effect when hypoplastic lungs were treated with the conditioned medium from amniotic fluid-derived stromal cells. Finally, intratracheal administration of human AFSCs can promote alveolarization in vivo in the surgical model of CDH.[104]

The mechanisms by which stem cells exert their beneficial effects on pulmonary tissue are still being investigated. Recently, AFSCs were shown to be able to restore epithelial cell homeostasis by attenuating the endoplasmic reticulum stress response in vitro in primary epithelial cells isolated from nitrofen-induced hypoplastic lungs.[105] Moreover, AFSCs release extracellular vesicles (AFSC-EVs) with a strong paracrine potential and regenerative effect.[106] We have recently shown that AFSC-EVs are the paracrine effectors executing the beneficial effects of AFSCs in an in vitro model of lung injury, as EV-depleted conditioned medium failed to show this regenerative potential.[107] AFSC-EVs represent a promising cell-free therapy in promoting lung growth and regeneration.

Cell-based therapies can also be used for engineering tissue for diaphragmatic replacement. Although prosthetic patches for large diaphragmatic defects repair have improved throughout the years, the ideal material has yet to be discovered as neonates that undergo a patch repair have a higher risk of CDH recurrence, infection, thoracic deformations, and small bowel obstruction than their counterparts.[108] Using tissue engineering to assemble a natural supporting scaffold seeded with stem cells is a promising novel strategy for diaphragmatic replacement, possibly avoiding these complications. A diaphragmatic tendon graft engineered with AF-MSCs recently achieved preclinical validation.[109]

Discussion and conclusion

A large body of basic scientific research on CDH exists and almost all studies include a statement emphasizing that the pathobiology is still not sufficiently understood. A combination of the individual epigenetic signature of the mother and fetus in combination with exposure to environmental factors at a certain developmental timepoint seems to underlie non-syndromic or isolated CDH.

To successfully improve CDH research, international and national collaborations are essential. Given the limited number of CDH cases in almost every academic center and different scientific backgrounds, collaboration is crucial to gain momentum in CDH research. Good examples like the Dhreams study group, the CDH Study Group, or individual collaborations exist and should be encouraged for further exploration.[74,110] We and other centers have established biobanks archiving CDH tissues or bio samples together with maternal and paternal samples.[111] Analyzing these human samples is necessary to allow translation of scientific findings from preclinical animal studies to human trials.

To advance our basic knowledge about CDH pathogenesis we need to leverage novel technologies (omics techniques) that allow for unbiased and broad investigations. Combining “omics” approaches with systems biology enables the investigation of all detectable molecules of a type (e.g. proteins, microRNA, mRNA) within one experiment and tissue sample. Subsequently, bioinformatic analysis can highlight biological processes and pathways that are dysregulated or enriched in pathological samples. This will focus CDH research on underlying biological patterns rather than single protein changes with limited consequences for the underlying biological disease. These omics approaches will be an invaluable source of information for the identification of novel CDH biomarkers to better prognosticate outcomes.[112]

The suspension of the STRIDER trial highlights the importance of careful and thorough testing of prenatally administered drugs in humans following promising results from preclinical animal studies. It is therefore mandatory that we adhere to the best possible scientific standards and ensure the highest transparency and open communication of our results.

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Conflict of interest

The authors declare no conflict of interest.

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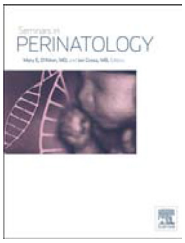

Long term follow-up in congenital diaphragmatic hernia

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ABSTRACT

Survivorship of patients with congenital diaphragmatic hernia (CDH) has created a unique cohort of children, adolescents and adults with complex medical and surgical needs. Morbidities specific to this disease benefit from multi-specialty care, and the long term follow up of these patients offers a tremendous opportunity for research and collaboration. Herein we aim to offer an overview of the challenges that modern CDH survivors face, and include a risk-stratified algorithm as a general guideline for a multi-specialty follow up program.

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Introduction

Congenital Diaphragmatic Hernia (CDH) is a complex, multisystem developmental anomaly with the diaphragmatic defect occurring in conjunction with possible cardiac, pulmonary, musculoskeletal and neurodevelopmental comorbidities. Overall survival in CDH has gradually improved in recent decades, largely due to focused research and the use of standardized postnatal management protocols with gentle ventilation strategies, extracorporeal membrane oxygenation (ECMO) utilization, and referral of patients to high-volume centers.[1–3]

The "price of success" however inherits a burden of increased morbidity for the modern survivorship of CDH. Today's CDH population is characterized by long term morbidity including chronic pulmonary dysfunction, a persistently reactive pulmonary vascular bed, neurodevelopmental challenges, hearing impairment, nutritional impediments and more (Fig. 1). This complex constellation of comorbidities is unique to survivors of CDH and is often predictable at initial hospital discharge.[4] Medical and surgical needs for CDH survivors include long term medications, home respiratory support, vasoactive medications, and often multiple subsequent operative interventions. These needs continue to affect newborn survivors through childhood, adolescence and adulthood, and late mortality related to progressive pulmonary insufficiency, pulmonary hypertension, and gastrointestinal complications after 1 year of life affect up to 13% of patients.[2]

Despite these challenges, patients and families with CDH uniformly agree that the benefit of a full life far surpasses the burden of medical morbidity, as patient- and parent-reported satisfaction with quality of life remains high.[5–9] Therefore, we have an opportunity to support these successes and herein we outline the key components of long-term follow up for these challenging, yet highly rewarding, survivors.

Role of a dedicated CDH long term follow up clinic

Individually, large tertiary referral centers with high CDH patient volumes have long recognized the necessity of long term follow up for CDH survivors. The first multi-disciplinary

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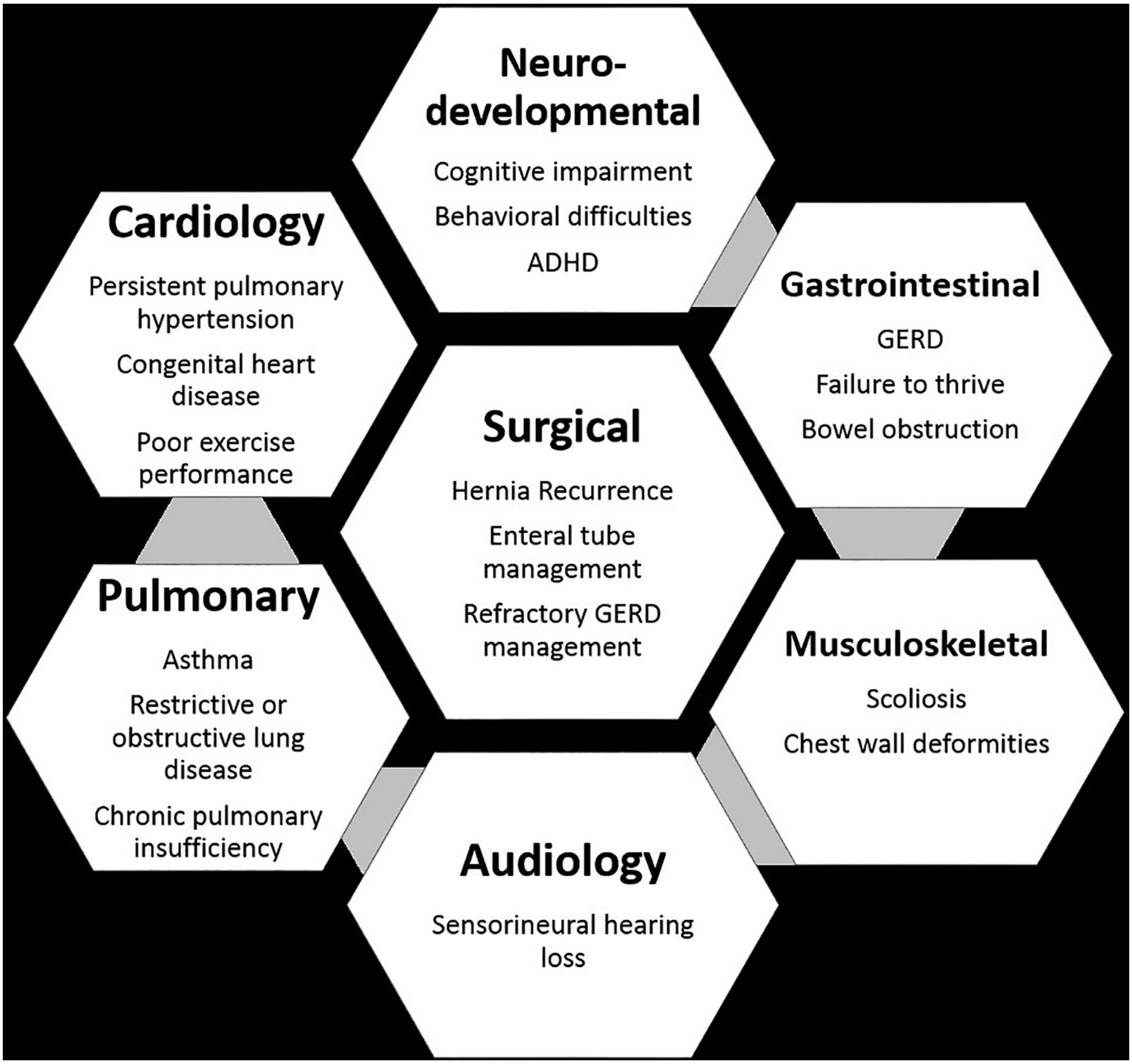

Fig. 1 – Long-term issues in CDH patients by system.

CDH clinic in the United States was established by Wilson and colleagues in 1990 and over time, many other centers across the world have emulated this model.[10,11]

Recognizing the importance of long term multi-specialty care, the American Academy of Pediatrics (AAP) first released a consensus statement in 2008 recommending guidelines for the structured multi-disciplinary follow up of survivors with CDH up to age 16.[12] These recommendations outlined a comprehensive multidisciplinary follow up plan specific to the unique physiology of patients with CDH. Recognizing this, as well as the unique opportunity for longitudinal data collection, the EURO Consortium proposed a collaborative project in 2018 using a standardized clinical assessment and management plan to facilitate international follow up for survivors of CDH.[13]

A large international interest exists in studying survivors of CDH as they mature into adulthood, and with more time and experience, increasingly more studies have been published.[14,15] Though open to interpretation and certainly variable to some degree, more uniform alignment on specific facets and scheduling for multi-specialty follow up is reflected in the literature to date. While specific protocols are not widely published, a general outline of key aspects to a multi-disciplinary follow up algorithm based on modern literature is described below.

Optimal components to a multidisciplinary long term CDH survivor clinic include pediatric surgery, neonatology, maternal-fetal specialists, ECMO team members, cardiology, pulmonology, neurology, audiology, gastroenterology, general pediatrics, dieticians, orthopedics, social work, and increasingly, telemedicine.[16] Coordination of these components remains challenging however, and not all centers can support a comprehensive multi-specialty clinic. Clinicians who care for these complex patients benefit from a template from which to model longitudinal follow-up for detection and subsequent management or referral for associated morbidities. Table 1 summarizes a compilation of previously published follow-up protocols[16] and stratifies patients by severity of CDH. In the following sub-sections, we will discuss key

Table 1 – Risk-stratified long-term follow up recommended schedule

	Before discharge	3 mo	6 mo	9 mo	12 mo	18 mo	24 mo then annually
Low Risk CDH: Type A/B, No supplemental O_2 requirement, no patch repair							
History and physical exam	X	X			X		X
Chest XR ± PFTs	X				X		
Cardiology f/u ± echo	X						
Neurodevelopmental	X						Formal testing at 3y and 5y
Brain imaging (MRI/CT)							
Gastrointestinal (UGI, pH probe)	As indicated	As indicated			As indicated		As indicated
Orthopedics (scoliosis, chest wall deformity screening)					X		X
Audiology							
High Risk CDH: Type C/D, on supplemental O_2, patch repair ECMO use							
History and physical exam	X	X	X	X	X	X	X
Chest XR ± PFTs	X	X	X	X	X	X	X
Cardiology f/u ± echo	X	X	X	As indicated	As indicated	As indicated	As indicated
Neurodevelopmental							Formal testing at 3y and 5y
Brain imaging (MRI/CT)	X						
Gastrointestinal (UGI, pH probe)	X	As indicated			As indicated		As indicated
Orthopedics (scoliosis, chest wall deformity screening)					X		X
Audiology	BAER testing	As indicated		As indicated			

attributes of the most impactful comorbidities affecting survivors of CDH.

Cardio-pulmonary follow-up

Medical management of patients with persistent pulmonary hypertension can be challenging, with the use of pulmonary vasodilators such as inhaled nitric oxide (iNO), sildenafil, and/or bosentan comprising most medications utilized in the current EURO CDH Consortium, American Heart Association and American Thoracic Society guideline recommendations.[3,17,18] The overall incidence of persistent pulmonary hypertension in the CDH population following initial hospital discharge appears to be between 10–20%.[19,20] While prenatally predicted severe pulmonary hypertension can persist throughout infancy, longitudinal studies demonstrate clinical and echocardiographic resolution over time in many.[20] This contradicts the classical thinking that all CDH associated pulmonary hypertension is irreversible; clearly there are postnatal factors that lead to improvement over time.

However, a subset of patients with severe CDH have persistent pulmonary hypertension that persists beyond infancy. In 2013, the World Symposium on Pulmonary Hypertension published screening, disease classification, and treatment algorithms with pediatric patients with pulmonary hypertension, including those with CDH.[21] These protocols focus on early identification and classification of pulmonary hypertension with screening echocardiograms and offer suggested guidelines for medical treatment protocols applicable to patients with CDH.

Patients with persistent pulmonary hypertension and pulmonary insufficiency typically include the more high-risk CDH population such as those born with the liver positioned up into the thorax and those who have a large enough defect to require a patch repair. In these high-risk survivors, their pulmonary function tests (PFTs) are often proportionately worse even into adolescence (14 years of age).[22] There may also be an association of worse PFTs in adolescence with a lower Body Mass Index (BMI), suggesting linkage to the nutritional morbidity that these patients may suffer and is discussed in further sections.[23]

Prenatal risk estimation and the relationship to long term pulmonary morbidity has yet to be clearly defined. In prenatally diagnosed infants with CDH who obtain observed to expected (O/E) lung-head circumference ratio (LHR) measurements, infants with moderately to severely diminished lung volumes have been shown to be at higher risk of perinatal demise.[24] However the long term prognostic value of ultrasonographic risk stratification with O/E LHR has not yet proven to be highly valuable or accurate, as risk stratification by this method has not correlated with pulmonary morbidity over time.[15]

Immediate postnatal risk factors for persistent pulmonary hypertension include longer duration of mechanical ventilation, longer hospital stays, and need for medical therapy at any time during hospitalization or at discharge.[25] Additional postnatal risk factors for long term pulmonary morbidity include the need for pulmonary support on day of life 30, which can predict morbidity at 1 and 5 years, specifically the need for supplemental oxygen and asthma.[26] However it should be noted that even patients who were on room air at day of life 30 have significant rates of inhaler use, V/Q mismatch, and asthma years after their initial hospitalization compared to healthy controls.[26]

Once survivors are discharged from the hospital, many will re-present with respiratory distress or wheezing within their first 2 years of life. In a French multi-center long term prospective study, nearly 60% of CDH survivors visited their doctor with wheezing before age 2 years and 40% were positive for respiratory syncytial virus (RSV) despite a 62% palizizumab prophylaxis rate.[27] Factors associated with readmission for wheezing correlate with those associated with persistent pulmonary hypertension such as liver-up position, prolonged index hospitalization, and need for oxygen therapy at home,[27] reflecting the common theme affecting pulmonary morbidity. Readmission rates in the United States within the first year of life for poor weight gain, respiratory distress, or fever occur in ~18%, even in patients who were considered to be lower risk and were on room air at day of life 30.[26]

Entering into childhood and adolescence, long term lung function may remain persistently below age matched controls. In a longitudinal study of 98 CDH survivors, children were found to lag behind expected results in both spirometry and fractional volumes until the age of 3 years, in particular if patch closure, ECMO support, or pulmonary vasodilators were required.[28] A long-term CDH follow up clinic in Croatia identified more than half of their survivors studied to have chronic symptoms or spirometric evidence of pulmonary disease, with lower peak oxygen consumption during cardiopulmonary exercise testing when compared with controls.[29] Exercise endurance time in children and adolescents may also be decreased at age 5, 8, and 12 years when compared to age-matched controls, particularly high-risk patients.[30] Of note, families must be cautioned of potential intolerance of high altitudes. Even children with CDH who are on room air at baseline can experience hypoxia in high altitude conditions such as flying.[31] Decreases in atmospheric pressure during flight can cause air in the gastrointestinal tract to expand by up to 30%, increasing pressure on both the abdominal and thoracic cavity, impeding respiratory function.[32] Caution with high-altitude exposures should be exercised with this at-risk population, and an altitude test simulating the hypoxic conditions of flying is available in pulmonary function labs. This can help assess the need for supplemental oxygen for safety when flying. This long-term morbidity further supports the need for long term pulmonology follow up of these high-risk patients.

Neurodevelopmental follow-up

Children affected by neonatal critical illness may incur known long-term neurodevelopmental consequences.[33] Prenatal brain imaging has not demonstrated significant differences in cerebellar morphology or brain parenchyma in fetuses with CDH compared to controls, even amongst nonsurvivors, suggesting that neurodevelopmental impairment is a postnatal consequence of therapy and not congenital.[34] The American Academy of Pediatrics Section on Surgery and the Committee on Fetus and Newborn recommends neurodevelopmental screening for infants with CDH starting at 9–12 months of age and then annually until age 5 years.[12] There are specific sub-populations of CDH survivors who may be at particularly increased risk for neurodevelopmental delay, including survivors who required ECMO support and those born prematurely.

Survivors of neonatal ECMO deserve particular attention. Longitudinal follow up of mixed populations of neonatal and pediatric survivors of ECMO demonstrates a wide range of neurological disabilities including cognitive testing decline, behavioral difficulties, and some degree of motor impairment compared to disease-matched controls without a history of ECMO.[35–37] This post-ECMO population has an overall spectrum of disability that persists over time, particularly cognitive ability, spatial tasks, behavior, hearing and motor function.[38–40] In a single institutional series in Austria, only 65% of ECMO survivors at age 6 years demonstrated a normal neurodevelopmental outcome with 35% demonstrating cognitive defects.[41] However quality of life scores of ECMO survivors consistently match similar to healthy peers.[36,37] In an intriguing review of young adult survivors of neonatal ECMO, these patients are satisfied with their lives, working and/or in college, in good health and starting their own families. These self-reported findings persist despite obstacles faced by survivors such as asthma, attention deficit disorder, learning difficulties and hearing problems, which are particularly problematic in the CDH population.[37]

Even without ECMO, survivors of CDH who required prolonged ventilation may be another subset of CDH survivors at particularly higher risk for cognitive and motor neurodevelopmental delay.[42] Younger gestational age may also predispose CDH survivors to adverse neurodevelopmental outcomes including cognitive, language and motor delay.[43] If screened at one year of age by an experienced developmental psychologist, over half of CDH survivors will exhibit at least a mild delay in at least on developmental domain.[44] This finding was associated with a low weight and head circumference growth trajectory, suggesting a simple screening tool to identify this subset of the CDH population that could benefit more from testing and potentially, intervention.[44,45]

Sensorineural hearing loss is also a significant contributor to neurodevelopmental morbidity of CDH survivorship. With a wide range of reported prevalence of 2.5–62%, sensorineural hearing loss may occur at a higher rate in CDH compared to other critically ill newborns (1–3%).[46,47] Auditory brainstem response or otoacoustic emissions screening before discharge and complete audiological surveillance are important adjuncts to screen and capture survivors who may benefit from therapeutic interventions as they mature into childhood.[47] Therefore, auditory screening prior to hospital discharge and during childhood remain an integral component of many centers' follow up algorithms.[16]

Though studies in CDH survivors suggest average overall intelligence, this population appears to be more at risk for working memory and attention deficits at school age.[48–50] These deficits tend to become more prominent with maturation and higher cognitive functioning requirements.[51–53] A substantially increased rate of emotional reactivity and autism-spectrum disorder has recently been described in survivors of CDH,[54] particularly those with major associated malformations and/or chromosomal anomalies. In those children, the relative risk of an autism-spectrum disorder was 9.3 times the general population.[55] These data encourage tailoring developmental follow up protocols and implementing early intervention in autism spectrum disorders to optimize long term outcomes.[56]

Interventions performed in childhood may facilitate overcoming neurological impairments incurred by neonatal critical illness. In a recent randomized study, survivors of CDH or neonatal ECMO aged 8–12 years demonstrated improvement in working memory and long-term visuospatial memory after Cogmed Working Memory Training, though some of the benefits declined over time.[45] This represents an intriguing area of potential therapy and research for future interventions in this high-risk population.

Gastrointestinal and metabolic follow-up

Failure to achieve growth goals is common and multifactorial in CDH, attributable to increased metabolic demand from increased work of breathing, gastroesophageal reflux disease (GERD), and oral aversion. Even when oral caloric intake is optimized, growth failure can persist. Aggressive screening and timely intervention with simple feeding protocols have proven to be useful to thwart growth inadequacy when CDH survivors are followed long term in a multidisciplinary setting.[57] Gastrostomy tubes are frequently placed in patients with CDH but are inconsistently effective in achieving growth velocity.[58,59]

Though the resting energy expenditure in CDH patients might be equivalent to predicted controls at hospital discharge if they have no respiratory support requirement,[60] patients with CDH nonetheless have long term difficulty meeting metabolic demands. Poorer lung function and lower BMI appear to correlate into childhood and adolescence.[23]

Gastroesophageal reflux disease (GERD) is also a significant contributor to morbidity and failure to meet growth targets. Esophageal dysmotility, weakness/absence of diaphragmatic crura, and changes in the anatomy required for diaphragm repair are major contributors to GERD in this population.[61] In a recent systematic review, GERD affected approximately 50% of infants with CDH and persisted beyond 1 year of life in 35%. When multichannel intraluminal impedance was performed the incidence was higher (60% beyond age 1 year).[62] Some institutions agree that impedance testing and pH measurements are critical for capturing subclinical reflux in this high risk population and recommend testing irrespective of overt symptoms in all CDH patients.[63] When surveyed endoscopically, a high prevalence of esophagitis and rarely, Barrett's esophagus, has been reported in patients with CDH in childhood and adolescence despite a low correlation with clinical symptoms.[64]

Poor linear growth can persist well after 1 year of age in CDH survivors, and a pattern of early deterioration of weight gain followed by a decline in linear growth can suggest inadequate nutrition during infancy.[65] Risk factors for poor growth velocity include need for home oxygen therapy and low birth weight,[66] but even patients considered to be low-risk such as those with a primary diaphragm repair are at risk for poor nutritional outcomes at the time of initial hospital discharge.[67] For these reasons, nutritional assessment and intervention should be initiated early on in follow up and continued throughout childhood.

Musculoskeletal follow-up

With advancements in modern critical care medicine, newborns can now undergo corrective thoracic surgeries for severe birth defects such as esophageal atresia, congenital heart disease, or congenital diaphragmatic hernia in infancy. Most CDH surgical repairs are trans-abdominal, and if the defect size is large, a patch can be required to achieve closure. Surgical closure may result in tension on the thorax, leading to the perhaps unforeseen sequelae of "thoracogenic scoliosis", or an acquired spinal deformity. Thoracogenic scoliosis has been reported in as many as 13–33% of children after CDH repair, 8.5% of children after cardiac surgery, and as many as 50% of children after tracheoesophageal fistula repair.[68,69] In the Japanese CDH Study Group registry survey of 159 survivors, 28% developed orthopedic anomalies including scoliosis, chest asymmetry and pectus excavatum.[70]

Survivors of CDH can benefit from early screening and referral to facilitate non-operative options for scoliosis management. Despite what common sense might hypothesize, patch repair may not be an independent predictor of development of scoliosis,[69] and some scoliosis may even be present congenitally in CDH.[69,71] In a recent series, 41% of patients with thoracogenic scoliosis required operative intervention,[68] emphasizing the need for early referral to our orthopedic colleagues as a means to improve efficacy of bracing programs.

In addition to scoliosis, CDH survivors may also acquire chest wall deformities such as chest asymmetry or pectus carinatum, particularly if a patch or muscle flap closure of the defect is required at birth.[72–74] Pectus excavatum affects up to 47% of survivors and appears to be more frequent than pectus carinatum (12%).[73] Patients with CDH can undergo successful minimally invasive repair of pectus excavatum defects caused by repair of the neonatal diaphragm and certainly would benefit of early screening and identification of the deformity.[75]

Needs specific to surgery

A wide range of morbidities related to surgical CDH repair has been reported for survivors, including postoperative small bowel obstruction, need for gastrostomy or fundoplication to address feeding difficulties, and diaphragmatic hernia recurrence.[76,77] Most of these surgical comorbidities occur within the first few years of life, but patients do benefit from long term surveillance.

In addition to defect size and overall patient risk profile, surgical morbidity is directly related to the method of repair. Open CDH repair, similar to open laparotomy for other diagnoses, incurs a known 5-fold increased risk of postoperative adhesive bowel obstruction in large CDHSG data series.[78] Up to 20% of CDH survivors may require operative intervention for a subsequent small bowel obstruction, and those at increased risk may include patients who required patch repair.[76]

With greater experience and comfort in technique, an increasing number of surgeons are choosing minimally invasive thoracoscopic CDH repairs for low-risk patients. In carefully selected patients with Type A (mild defect surrounded by muscle) or Type B (< 50% portion of the chest wall devoid of muscle) defects, thoracoscopic repair can be associated with reduced hospital length of stay, less need for mechanical ventilation, and earlier initiation of feeding even when stratified by defect size.[79]

Many studies have echoed a higher recurrence rate for thoracoscopic repairs however. A modern review of the CDH Study Group registry data including 3067 patients demonstrated early recurrence rates of 5.9% with the thoracoscopic repair versus 3.2% in open repairs during the initial hospitalization.[78] However more extended long term patient follow up studies have demonstrated recurrence for minimally invasive repairs to range from 6−39% compared to 0−13% for the open approach.[80,81] The use of a minimally invasive approach and larger defect size both appear to be factors associated with early hernia recurrence.[82]

Regardless of method of repair, operative intervention for CDH recurrence is a technically challenging intervention best managed in a center with medical and surgical experience with these complex patients. Families should be counseled by the surgeon regarding specific signs and symptoms of bowel obstruction and if they develop, timely evaluation and intervention should be undertaken.

Impact on the family

While our purpose is to focus on the medical and surgical needs of our CDH survivors, we would be remiss to omit the significant effect of chronic illness and emotional stress on these patients and their families. Even up to 8 years of age, these families bear the burden of medical equipment (62%), home health services (18%), and special educational needs (28%). However when queried, health care related quality of life (HRQOL) does not differ greatly from other healthy children controls or less severe CDH, echoing the findings of many other studies.[5−9] The Family Impact score demonstrates that most parents report a fairly low burden of their child's illness over time, however 68% are "often or always worrying about their child" due to their health condition. The need for special education and an increased Family Impact Score largely related to neonatal CDH can diminish the overall HRQOL however.[6]

In the perinatal period, parents of babies with complex surgical needs are often overwhelmed with the breadth of medical and surgical problems and can exhibit signs of posttraumatic stress disorder.[83−85] Confounding this parental stress can be the many different objectives of multiple teams of providers caring for their babies. This can create deep and lasting distress and the balance of wanting information can be overturned by feelings of hopelessness and loss. Helpful adjuncts reported by parents of survivors include parental support groups both in person and within social media outlets as well as online interest groups.[86,87]

Unique long-term morbidities of prenatal intervention

Fetuses with CDH have historically been a focus for prenatal or early perinatal intervention. Early attempts at fetal diaphragm repair with open maternal-fetal surgery (OMFS) ultimately demonstrated no survival advantage for the fetus with CDH, however several significant technical progresses were made, leading to advances in fetal surgery and subsequent maternal management for other diagnoses.[88] The ex-utero intra-partum treatment (EXIT)-to-ECMO strategy was examined as a means to immediately transfer the newborn infant from placental support to ECMO support at birth, thus avoiding a critical postnatal period of respiratory failure characterized by profound hypoxemia, acidosis and instability. Unfortunately, the EXIT-to-ECMO procedure does not appear to confer a survival benefit or decrease long-term pulmonary, cardiac, or neurodevelopmental morbidity for fetuses with severe CDH.[89,90]

A novel prenatal intervention, fetal endoscopic tracheal occlusion (FETO), was designed to overcome the congenital pulmonary hypoplasia induced by a severe CDH. FETO is a minimally invasive technique in which a tracheal occlusion balloon is placed endoscopically into a fetus, percutaneously under local anesthesia. Occlusion of the fetal trachea leads to fluid accumulation in the fetal lungs, increasing airway pressure, cellular proliferation, and improving pulmonary airspace and vasculature maturation.[91] The tracheal balloon is typically inserted at about 27−30 weeks and removed 6 weeks later prior to delivery. Exciting early experimental results have conferred a perinatal survival benefit of FETO to carefully selected fetuses with severe CDH.[92,93] Long term data on FETO survivor are scarce, but in a recent publication including 10 survivors of FETO, morbidity at 1 year of age was primarily related to GERD and gastrostomy tube dependence, not pulmonary hypoplasia. These patients did have a high hospital readmission rate (56%) related to respiratory symptoms however, and 1 in 4 were noted to have persistent tachypnea and need for chronic inhalational therapy.[94] The Tracheal Occlusion to Accelerate Lung Growth, or TOTAL trial, is an active international multi-center randomized controlled trial including fetuses with severe or moderate CDH. Enrollment for patients with moderate CDH has recently ended as of May 2019, and forthcoming results will hopefully provide information of short term and long term survival benefits (www.totaltrial.eu, accessed 5/6/2019).

Hundreds of mothers and babies around the world have now undergone the FETO procedure for severe or moderate CDH. Procedure-related maternal complications include premature rupture of membranes and preterm delivery[95] and babies who undergo tracheal balloon placement are noted to have tracheomegaly both at birth[96] and at a longer duration of follow-up.[97] Fortunately, tracheomegaly confers few to no additional clinical harm or implications at a mean follow-up age of 34 months.[97]

The first reports of maternal outcomes, particularly concerning future pregnancies, are just now emerging. In a survey series of 89 women who underwent FETO prenatal intervention for CDH, 70% attempted a second pregnancy and none reported barriers to future fertility. When matched to controls, there were no differences in rate of future conception (approximately 90%) or rate of first trimester pregnancy loss. No women experienced middle trimester pregnancy losses and the rates of subsequent preterm deliveries were equal between groups.[98] Importantly, fetoscopy did not place the mothers at risk for uterine scar problems with subsequent pregnancies, which is quite different compared to OMFS intervention, in which the rate of uterine scar dehiscence and rupture can approach 14%.[99]

Transition of care to adult clinicians

There have been a substantial number of recent studies describing the outcomes of congenital anomalies into adulthood, reflecting a growing interest of clinicians in the transition of care and long-term outcomes.[100−104] Nevertheless, there is no standard recommendation for pediatric surgical patient follow up for many patients surviving with repaired congenital anomalies, such as esophageal atresia/tracheoesophageal fistula, anorectal malformations, and CDH. Our colleagues within pediatric cardiology and cardiac surgery face the same dilemma with an increased number of adult congenital heart disease survivors leading to an influx of long term follow-up needs and specialized centers across the country.[105] Perhaps future endeavors can focus on identifying long term solutions that will provide optimal care for these patients and potentially reduce the burden on the health care system.

Conclusion

Our growing population of CDH survivors carry a burden of chronic health conditions across a wide spectrum of pediatric specialty care. Despite this morbidity, it is important to recognize that many patients describe themselves as healthy, symptom-free and without limitations. Practice standards are currently evolving to emulate centers that provide longitudinal, multi-disciplinary comprehensive care for these challenging, yet highly rewarding patients.

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